(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 July 2006 (13.07.2006)

PCT

(10) International Publication Number
WO 2006/072957 A1

(51) International Patent Classification:
A61K 31/343 (2006.01) *A61K 31/397* (2006.01)
A61K 31/18 (2006.01) *A61P 25/28* (2006.01)
A61K 31/445 (2006.01) *A61P 37/00* (2006.01)
A61K 31/49 (2006.01)

(21) International Application Number:
PCT/IL2006/000027

(22) International Filing Date: 5 January 2006 (05.01.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
166149 5 January 2005 (05.01.2005) IL

(71) Applicant *(for all designated States except US)*: **HADASIT MEDICAL RESEARCH SERVICES & DEVELOPMENT LTD.** [IL/IL]; P.O. Box 12000, 91120 Jerusalem (IL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **ROSENMANN, Hanna** [IL/IL]; 10/1 Theodore Lavi Street, Ramot, 97281 Jerusalem (IL).

(74) Agents: **LUZZATTO, Kfir** et al.; P.O. Box 5352, 84152 Beer Sheva (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/072957 A1

(54) Title: ANTI-ARRYTHMIC DRUGS AND CHOLESTEROL ABSORPTION INHIBITORS AS NEUROPROTECTIVE AGENTS FOR THE TREATMENT OF NEURODEGENERATIVE AND AUTOIMMUNE DISORDERS

(57) Abstract: The present invention relates to a method for the treatment or prevention of a neurodegenerative disorder or an or an autoimmune-related disorder, particularly, CNS inflammatory autoimmune disorder, by administering to a subject in need thereof, a therapeutically effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof. The invention further relates to the use of an antiarrhythmic agent and/or a cholesterol absorption inhibitor agent in the preparation of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder or an autoimmune related disorder. A method for protection of neuronal cells from a neurodegenerative process, is also provided by the invention.

ANTI-ARRYTHMIC DRUGS AND CHOLESTEROL ABSORPTION INHIBITORS AND NEUROPROTECTIVE AGENTS FOR THE TREATMENT OF NEURODEGENERATIVE AND AUTOIMMUNE DISORDERS

Field of the Invention

The present invention relates to use of antiarrhythmic agents, particularly amiodarone and of cholesterol absorption inhibitor agents, particularly ezetimibe, in the treatment of neurodegenerative disorders and CNS related inflammatory autoimmune disorders.

Background

All publications mentioned throughout this application are fully incorporated herein by reference, including all references cited therein.

Neurodegenerative disorders, which are chronic and progressive, are characterized by selective and symmetric loss of neurons in motor, sensory, or cognitive systems. Delineation of the patterns of cell loss and the identification of disease-specific cellular markers have aided in nosologic classification, senile amyloid plaques (SP), neurofibrillary tangles (NFT), neuronal loss, and acetylcholine deficiency define Alzheimer's disease (AD), Lewy bodies and depletion of dopamine characterize Parkinson's disease, cellular inclusions and swollen motor axons are found in amyotrophic lateral sclerosis, and γ-aminobutyric acid–containing neurons of the neostriatum are lost in Huntington's disease.

It is well accepted that the intrinsic differences in cellular metabolism of distinctive brain regions and neuronal populations underlie the selective cellular response to various environmental neurotoxicants, and neuropathological conditions, therefore causing distinct neurodegenerative diseases.

Amyloid diseases are caused by the misfolding of proteins into structures that lead them to cluster together, forming microscopic fibril or plaques, which deposit in internal organs and interfere with normal function, sometimes lethally.

These diseases include Alzheimer's disease, Parkinson's disease, and the peripheral nervous system disease familial amyloid polyneuropathy (FAP). In Alzheimer's disease, these clumps are termed amyloid plaques and consist primarily of the amyloid-beta (Aβ) peptide. In the case of Alzheimer's disease, these fibrils cause degeneration of nerve cells in areas of the brain that are crucial for memory. The Aβ peptides possess neurotoxic properties also in their soluble form. In Parkinson disease, they are called Lewy bodies and contain the protein α-synuclein. FAP, a collection of more than 80 rare amyloid diseases are caused by the misfolding of the protein transthyretin (TTR), which the liver secretes into the bloodstream to carry thyroid hormone and vitamin A.

In the FAP diseases, mutations in the TTR protein are known to play a direct role in causing the disease. These changes alter protein folding in such a way as to predispose the proteins to misfold and accumulate into microscopic fibrils, which can grow into protein plaques [Reixach N. et al., (2004) Proc. Natl. Acad. Sci. U S A. 101: 2817-2822].

In Alzheimer's disease, the cause of misfolding is not so obvious. A number of mutations are associated with rare forms of familial Alzheimer's disease, but not with most common cases (about 95 percent of the cases). This suggests there must be a more common cause of Alzheimer's disease.

Traumatic head injuries are a major risk factor for later developing Alzheimer's disease. The body responds to such injuries with

inflammatory reactions that cause the release of components of lipid membranes, such as cholesterol. Inflammation can lead to the production of reactive oxygen species such as ozone, which can trigger pathological changes in other molecules in the body, like cholesterol.

Common risk factors have been found between atherosclerosis and neurodegenerative diseases, including hypercholesterolemia and inflammation. Inflammation in the brain could create a perfect storm in which cholesterol and lipids react with ozone and other inflammatory chemicals to produce abnormal reactive metabolites, which, in turn, modify the folding of normal Aβ peptide. These modified Aβ peptides can catalyze misfolding in other unmodified Aβ peptides, starting an avalanche of misfolding that may result, perhaps years or decades later, in Alzheimer's disease [Zhang Q. et al., (2004) Proc. Natl. Acad. Sci. U S A. 101: 4752-4757].

This theory implies the creation of a reactive metabolite by inflammatory stress, which leads to the modification of a protein, the aggregation of that protein over time, and the degeneration of function in the brain or whichever internal organ hosts the aggregation.

Alzheimer's disease (AD) is a progressive neurodegenerative incurable disease. It is the major cause of dementia in the elderly. The estimated number of patients is approximately 20 million worldwide and is expected to keep growing as the world population ages. In the USA, an estimated 10% of Americans over the age of 65 and half of these over 85 have AD. AD is now the third most expensive disease to treat in the USA, costing society close to $100 billion annually.

The onset of the disease is characterized by impaired memory but with disease progression other intellectual skills decline. Later, erratic

behavior, delusions and a loss of control over body functions occur. The major brain pathological features include the senile amyloid plaques (SP), composed of Aβ peptide, and the neurofibrillary tangles (NFT), which are aggregations of the hyperphosphorylated microtubular protein tau.

Cholinergic dysfunction as well as oxidative stress are implicated in the disease pathogenesis. As these cellular changes progress, neurons are lost in the hippocampus, entorhinal cortex, and association areas of the neocortex [reviewed in Mayeux R. (2003) Annu. Rev. Neurosci. 26:81-104; Selkoe D. (2001) Physiological Rev. 81:741-766].

The etiology of AD is complex and involves a combination of factors including genetic, immune, endocrine and environmental factors. One such AD-related factor that is attracting recently great attention, is the role of cholesterol metabolism and trafficking. There is accumulating data in support of the hypothesis that altering in the cholesterol levels influences the development of AD by affecting the formation of Aβ peptide, its distribution within cholesterol rich membranes and its fibrillogenesis.

Cultured human cells exposed to high cholesterol levels showed a four-fold increase in Aβ peptide synthesis [Stephens D. et al., (1999) Neuroreport 10:1699-1705]. Aβ peptide toxicity to vascular smooth muscle cells correlates strongly to the amount of cholesterol in the cell membranes [Subasinghe S. et al., (2003) J. Neurochem. 84:471-479]. The pathogenic fibrillogenesis of Aβ peptide has been shown to be cholesterol dependent in cell cultures [Mizuno T. et al., (1999) J. Biol. Chem. 274:15110-15114].

In a transgenic mouse model presenting the amyloid pathology of AD (overexpresses mutant amyloid precursor protein and presenilin-1) a high cholesterol diet results in significantly increased levels of Aβ peptide in brain tissue, with both the number and size of Aβ peptide deposits were

elevated. Levels of total Aβ peptide were strongly correlated with the levels of both plasma and CNS total cholesterol [Refolo L. et al., (2000) Neurobiol. Dis. 7:321-731]. When treating these mice with cholesterol lowering agents, plasma cholesterol, brain Aβ peptides and plaques load decreased [Refolo L. et al., (2001) Neurobiol. Dis. 8:890-899].

Taken together, these results strongly support the view that cholesterol is responsible for Aβ peptide formation and toxicity, although it is not clear that disruption of cholesterol balance is a primary cause of AD rather than a secondary effect of the disorder.

As for cholesterol in humans, epidemiological studies indicate that there is a decreased prevalence of AD associated with the use of cholesterol-lowering statins [Wolozin B. et al., (2000) Arch. Neurol. 57:1439-1443; Xavier A. et al., (2003) CNS Drugs 17:213-224].

Although clinical benefits are largely attributed to their lipid-lowering properties, there is much debate regarding the contribution of the plieotropic effects of statins to AD. Statins display additional cholesterol independent effects on cerebral circulation and inflammation, effects that may be beneficial in AD, while some other effects may not.

Inhibition of cholesterol absorption may be neuroprotective against Aβ peptide neurotoxicity. For this purpose the inventors examined the cholesterol absorption inhibitor, ezetimibe (Ezetrol), on Aβ peptide neurotoxicity. Ezetimibe is a newly used medication in cardiovascular diseases for lowering cholesterol. It is a selective and very effective 2-azetidione cholesterol absorption inhibitor which acts at the level of cholesterol and related plant sterols entry into enterocytes [Van Heek M. et al., (1997) J. Pharmacol. Exp. Ther. 283:157-163] and lowers the amount of cholesterol in the body and the blood. Ezetimibe inhibits the

uptake and/or the processing of the low-density lipoprotein cholesterol (LDL-C), and affects the class B scavenger receptor mediated uptake pathway required for receptor mediated endocytosis of LDL-C [Seedorf U. et al., (2004) Biochem. Biophys. Res. Commun. 320:1337-1341]. Ezetimibe is orally active, and has a mechanism of action that differs from other classes of cholesterol-reducing compounds [e.g. statins, bile acid sequestrants (resins), fibric acid derivatives, and plant stanols].

Ezetimibe localizes at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. Statins reduce cholesterol synthesis in the liver and together, these distinct mechanisms provide complementary cholesterol reduction. Ezetimibe by itself does not inhibit cholesterol synthesis in the liver neither increases the bile acid secretion.

After oral administration, ezetimibe is rapidly absorbed and extensively conjugated to a pharmacologically-active phenolic glucuronide (ezetimibe-glucuronide). Ezetimibe and ezetimibe-glucuronide half-life is approximately 22 hours.

The neuroprotective effect of different compounds including ezetimibe on PC-12 cell line, a neuronal-cell culture model, exposed to Aβ peptide neurotoxicity was examined by the present inventors. PC-12 cell culture is a rat pheochromocytoma cell line, which displays phenotypic characteristics of sympathetic neurons. In the past decade, different cellular and molecular experiments have shown that PC-12 cells are an excellent *in vitro* model for investigating various neurological disorders, such as Alzheimer's disease and Parkinson's disease [Troy C. et al., (2001) J. Neurochem. 77:157-164; Rideout H. et al., (2001) J. Neurochem. 78:899-908; Shimoke K. and Chiba H. (2001) J. Neurosci. Res. 63:402-409].

PC-12 cells die in a dose dependent manner in response to exposure to the AD-related Aβ peptide. These cells also suffer from the neurotoxic effects when coming in contact with high cholesterol levels, or under serum–deprivation conditions. On the other hand, these cells benefit from the neuroprotective effect of statins, vitamin E and vitamin C after exposure to the neurotoxic effect of Aβ peptide. The neuroprotective effects of statins, vitamin E and vitamin C, have been previously reported in epidemiological studies and in clinical trials [Zamrini E. et al., (2004) Neuroepidemiology 23:94-98; Zandi P. et al., (2004) Arch. Neurol. 61:82-88].

PC-12 cell culture is therefore, a convenient model to test neurotoxicity and to detect neuroprotective drugs capable of overcoming the effects of such toxic conditions. The neuroprotective effect of different tested compounds on neuronal cells cultured in the presence of Aβ peptide neurotoxic is reflected by the increment of the neuronal cell survival.

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, with a prevalence of two percent among people over the age of 65 years. The disease is mostly sporadic, but familial forms are recognized as well. Parkinson disease (PD) targets dopaminergic neurons in the substantia nigra, resulting in motor disturbances such as resting tremor, bradykinesia, and rigidity. There is a substantial clinical overlap between Alzheimer's disease and Parkinson's disease. Dementia develops in approximately 20 to 30 percent of patients with Parkinson's disease, and the brains of these patients often contain Lewy bodies, SP and NFT.

The third common neurodegenerative diseases are the motor neuron diseases. The most common motor-neuron disorder is amyotrophic lateral sclerosis (ALS), which usually begins in the fifth and sixth decades of life.

The illness is usually sporadic, but in 1 to 10 percent of patients it is familial, being inherited as an autosomal dominant trait. In a typical patient, muscles innervated by both brain stem and spinal cord atrophy as lower motor neurons die, although those that control eye movements and bowel and bladder function are spared. The prognosis is grave, with death occurring in three to five years in 95 percent of patients.

Another neurodegenerative disease is the Huntington's disease, which is an autosomal dominant disorder with high penetrance. The characteristic findings of progressive chorea and dementia are caused by severe neuronal loss, initially in the neostriatum and later in the cerebral cortex. Although the regions and cells that degenerate in these various illnesses and insults are distinct, several features are common to many of these conditions and include aberrant protein interactions and aggregation, mitochondrial dysfunction, altered antioxidant defenses, oxidative stress, inflammation and apoptosis.

Different neurological disorders, known as "taupathies" have been recently described. In these disorders it has been suggested that modifications in the microtubule-associated protein tau could cause neural degeneration in specific regions. Although these regions are different in the different taupathies, some common features appear to occur in all of them: neurofibrillary tangles (NFT), which are aggregations of the abnormal hyperphosphorylated microtubular protein tau.

Abnormal tau proteins are often seen as mechanisms that can lead to brain degeneration in Alzheimer's disease and other neurodegenerative disorders known as taupathies. In all taupathies, there are neuropathologic aggregates of paired helical filaments and/or straight filaments composed of aberrantly phosphorylated tau proteins in central nervous system neurons or glia.

Although impressive advances in understanding of these diseases have been made, still the mechanisms of brain degeneration are not resolved, and no effective drug is available. To date only the secondary degenerative effects have been amenable to therapy.

The present invention describes a detailed examination of various candidate compounds, particularly, antiarrhythmic drugs, for their neuroprotective potential under neurotoxic conditions. These compounds were analyzed in neuronal cell culture model (PC-12), and in animal models.

Amiodarone binds to sodium, potassium, and calcium channels in addition to being a beta-blocker. It is one of the most powerful antiarrhythmic medicines in suppressing arrhythmias. It predominantly blocks potassium channels and prolongs repolarization, but amiodarone also induces noncompetitive alpha- and beta-adrenergic receptor antagonism, calcium channel inhibition and changes in the thyroid hormone metabolism. In the Vaughan Williams classification of antiarrhythmics, amiodarone is considered to be a predominantly class III agent, with some class I properties.

In addition to being an antiarrhythmic medication, amiodarone also causes blood vessels to dilate (enlarge). This effect can result in a drop in blood pressure. Because of this effect it also may be of benefit in patients with congestive heart failure.

The neuroprotective effect of amiodarone detected in cell culture as well as in animal models, was further explored by the inventors for investigating its potential neuroprotective effect in animal models for diseases of the central nervous system (CNS). Therefore, the inventors examined the effect of amiodarone on experimental autoimmune

encephalomyelitis (EAE), an autoimmune inflammatory disease resulting in demyelination of the white matter in the CNS. In many of its clinical and histopathological aspects, EAE resembles human multiple sclerosis (MS) and acute disseminating encephalomyelitis. EAE can be induced in genetically susceptible animals by a single s.c. injection of myelin associated antigens, such as myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP), emulsified in CFA and followed by a booster with Bordetella pertussis. A characteristic monophasic paralytic disease develops 10-13 days later. EAE serves as a useful experimental model for investigating new therapeutic strategies in MS. Various immunosuppressive agents were found effective in prevention and treatment of EAE, including corticosteroids and copolymer 1. However, patients are so far treated either symptomatically or with immunosuppressive agents, and no satisfactory therapy for MS has as yet been established.

It is therefore an object of the invention to provide methods using ezetimibe, amiodarone and like compounds in the treatment of subjects suffering from decreased mental and physical functioning, particularly AD, PD, ALS, HD, taupathies and prion related disorders as well as from autoimmune diseases, particularly inflammatory diseases related to the central nervous system (CNS), such as MS, which dramatically reduce the quality of life of the patients, and increase the burden on the family and caregivers.

The advantage of the methods of the invention lies in the opportunity to use ezetimibe and/or amiodarone as well as other known antiarrhythmic drugs, which are already medically approved and are in use for treatment of other conditions (mostly cardiovascular and heart diseases), giving hope to already-affected and to potentially-affected patients.

Summary of the Invention

In a first aspect, the invention relates to a method for the treatment or prevention of a pathologic disorder, in a subject in need thereof. The pathologic disorder treated by the invention may be selected from neurodegenerative disorders and autoimmune-related disorders. According to one embodiment, the method of the invention comprises the step of administering to a subject in need, a therapeutically effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof.

In one preferred embodiment, the method of the invention comprises the step of administering to a subject in need thereof, a therapeutically effective amount of an antiarrhythmic agent or of a composition comprising the same. More particularly, the antiarrhythmic agent may be any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof. Preferably, the antiarrhythmic agent used by the method of the invention may be amiodarone or any active derivatives thereof.

In another alternative and preferred embodiment, the method of the invention comprises the step of administering to a subject in need thereof, a therapeutically effective amount of a cholesterol absorption inhibitor agent, preferably, ezetimibe, or of a composition comprising the same.

In a second aspect, the invention relates to the use of an antiarrhythmic agent and/or a cholesterol absorption inhibitor agent in the preparation of a pharmaceutical composition for the treatment or prevention of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune related disorder, in a subject in need thereof.

In a third aspect, the present invention relates to a method for protection

of neuronal cells from a neurodegenerative process. The method of the invention comprises the step of contacting these neuronal cells with a neuroprotective effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof.

According to one embodiment, the neurodegenerative process may be caused by a neurodegenerative disorder in a mammalian subject. For example, a protein misfolding disorder, an amyloid disease or a prion disease. Alternatively, the neurodegenerative process to be prevented by the method of the invention may be caused by exposure of the neuronal cells to a neurotoxic agent.

The present invention further provides a method of preparing a therapeutic composition for the treatment of a pathologic disorder such as, a neurodegenerative disorder or an autoimmune related disorder, in a mammalian subject. This method comprises the steps of: (a) providing any one of an antiarrhythmic agent and a cholesterol absorption inhibitor agent; and (b) admixing the antiarrhythmic agent, the cholesterol absorption inhibitor agent or any combination thereof, with at least one of a pharmaceutically acceptable carrier, diluent, excipient and/or additive.

Brief Description of Figures

Figure 1A-1B: PC-12 neuronal cells response to neurotoxic and neuroprotective agents

Fig. 1A: *PC-12 neuronal cells respond to neurotoxic conditions*

Cell survival (%) of cell exposed to 1μM Aβ (25-35) peptide and 5μM cholesterol neurotoxic substances, or of the cells grown in a serum free medium neurotoxic condition, was compared to control cells not exposed to

neurotoxic substances or neurotoxic conditions. The p value was calculated relative to the control cells.

Fig. 1B: *PC-12 neuronal cells respond to neuroprotective conditions*

Cell survival (%) of cell exposed to 1μM Aβ (25-35) peptide (neurotoxic substance) and treated with 1μM simvastatin or with 100μM vitamin E together with 100μM vitamin C (neuroprotective substances), was compared to control cells not exposed to 1μM Aβ (25-35) peptide or to any treatment. The p value was calculated relative to cells exposed to 1μM Aβ (25-35) without any further protective treatment. Abbreviations: con. (control), ce. (cell), sur. (survival), SF. (serum free).

Figure 2: Treatment of PC12 cells exposed to Aβ peptide (model of AD), with different concentrations of ezetimibe

PC-12 cells exposed to 1μM Aβ (25-35) peptide were treated with different concentrations of ezetimibe. Cell survival (%) was calculated relative to control cells not exposed to the Aβ peptide or to ezetimibe. The p value was calculated relative to cells exposed to the Aβ peptide, but not treated with ezetimibe. Abbreviations: con. (control), ce. (cell), sur. (survival).

Figure 3: Treatment of PC12 cells exposed to high-cholesterol, with ezetimibe

PC-12 cells exposed to high-cholesterol toxicity (5μM) were treated with 100μM ezetimibe. Cell survival (%) was calculated relative to control cells not exposed to high-cholesterol or to ezetimibe. The p value was calculated relative to cells exposed to high-cholesterol without any further ezetimibe treatment. Abbreviations: con. (control), ce. (cell), sur. (survival).

Figure 4: Treatment of PC12 cells exposed to Aβ peptide (model of AD), with different concentrations of amiodarone

PC-12 cells exposed to 1μM Aβ (25-35) peptide were treated with different concentrations of amiodarone. Cell survival (%) was calculated relative to

control cells not exposed to the Aβ peptide or to amiodarone The p value was calculated relative to cells exposed to the Aβ peptide, but not treated with amiodarone. Abbreviations: con. (control), ce. (cell), sur. (survival).

Figure 5A-5D: Amiodarone shows neuroprotection in PC12 neuronal cell cultures against 3-NP (model of HD), MPTP and 6-OHDA (models of PD), and glutamate (model of ALS)

PC-12 cells exposed to four different neurotoxins were treated with different concentrations of amiodarone. Cell survival (%) was calculated relative to control cells not exposed to the neurotoxin or to amiodarone. The p value was calculated relative to cells exposed to the neurotoxin, but not treated with amiodarone.

Fig. 5A: Treatment of PC12 cells exposed to 6-hydroxydopamine (6OHDA) as a neurotoxin, with different concentrations of amiodarone.

Fig. 5B: Treatment of PC12 cells exposed to 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), as a neurotoxin, with different concentrations of amiodarone.

Fig. 5C: Treatment of PC12 cells exposed to 3-nitropropionic acid (3-NP) as a neurotoxin, with different concentrations of amiodarone.

Fig. 5D: Treatment of PC12 cells exposed to glutamate as a neurotoxin, with different concentrations of amiodarone. Abbreviations: con. (control), amio (amiodarone).

Figure 6A-6D: Pre-treatment with amiodarone reduces the PD symptoms as demonstrated by a PD-animal model

Rats stereotaxically injected with 6OHDA into the right substantia nigra developed PD-related motor deficits, starting 3 days following injection. These rats were used for examining the neuroprotective effect of amiodarone.

Fig. 6A: Rats were pre-treated with 30-40 mg/kg amiodarone in drinking water starting 4 weeks before disease induction (6OHDA injection), and

continuing until the end of experiment. Motor performance was evaluated using the stepping test.

Fig. 6B: Rats were pre-treated with 30-40 mg/kg amiodarone in drinking water starting 4 weeks before disease induction (6OHDA injection), and continuing until the end of experiment. Motor performance was evaluated using the placing test.

Fig. 6C: Rats were pre-treated with 20 mg/kg amiodarone injected i.p. starting 2 weeks before disease induction (6OHDA injection), and continuing until the end of experiment. Motor performance was evaluated using the stepping test.

Fig. 6D: Rats were pre-treated with 20 mg/kg amiodarone injected i.p. starting 2 weeks before disease induction (6OHDA injection), and continuing until the end of experiment. Motor performance was evaluated using the placing test.

Abbreviations: con. (control), trea. (treatment), si. (side), D. (days), po. (post), ind. (induction).

Figure 7A-7B: Stopping amiodarone treatment deteriorates PD symptoms

Rats stereotaxically injected with 6OHDA into the right substantia nigra developed PD-related motor deficits, starting 3 days following injection. These rats were used for evaluating the neuroprotective potential of amiodarone, by examining the effect of stopping amiodarone treatment. In this experiment, the treatment with 30-40 mg/Kg amiodarone in drinking water, starting 4 weeks before disease induction (experiment of figure 6A-B), was stopped at day 50 following disease induction.

Fig. 7A: Stopping the treatment with amiodarone (30-40 mg /kg in drinking water) at day 50 following disease induction. Motor performance was evaluated using the stepping test.

Fig. 7B: Stopping the treatment with amiodarone (30-40 mg /kg in drinking water) at day 50 following disease induction. Motor performance was evaluated using the stepping test.
Abbreviations: con. (control), trea. (treatment), si. (side), D. (days), po. (post), ind. (induction).

Figure 8A-8C: Treatment with amiodarone by i.p. injections as well as by gavage ameliorates the PD symptoms in animal model
Rats stereotaxically injected with 6OHDA into the right substantia nigra developed PD-related motor deficits and therefore serve as a PD model. These rats were used for examining the neuroprotective effect of amiodarone starting at day of disease induction, administered using different methods.
Fig. 8A: Rats were treated with 20 mg/kg amiodarone injected i.p. from day of disease induction. Motor performance was evaluated using the stepping test.
Fig. 8B: Rats were treated with 20 mg/kg amiodarone injected i.p. from day of disease induction. Motor performance was evaluated using the placing test.
Fig. 8C: Rats were treated by gavage of 75-100 mg/kg amiodarone from day of disease induction. Motor performance was evaluated using the stepping test.
Abbreviations: con. (control), trea. (treatment), si. (side), D. (days), po. (post), ind. (induction).

Figure 9: Treatment with amiodarone i.p. at day 70 post disease induction ameliorates the PD symptoms
Rats stereotaxically injected with 6OHDA into the right substantia nigra serve as a PD model. These rats were used for examining the neuroprotective effect of 50mg/kg amiodarone, administered i.p. at day 70 post disease induction. Motor performance was evaluated using the

stepping test. Abbreviations: con. (control), la. (late), trea. (treatment), si. (side), D. (days), po. (post), ind. (induction).

Figure 10A-10C: Pretreatment with amiodarone in drinking water delays ALS-disease onset and death in ALS-animal model

Pretreatment of ALS tg mice with 30-40 mg/kg amiodarone in drinking water delays the ALS-disease onset and delays death (prolongs survival) compares to that of the control ALS mice.

Fig. 10A: shows measured performance of healthy animals on rotarod.

Fig. 10B: shows clinical score (score 4 = no clinical symptoms).

Fig. 10C: shows delay in the death as reflected by survival (score >0)

Abbreviations: con. (control), trea. (treated), D. (days), ca. (cases), ag. (age), W. (weeks).

Figure 11A-11B: Sotalol shows neuroprotection in PC12 neuronal cell cultures against Aβ peptide (model AD) and against 3-NP (model of HD)

PC-12 cells exposed to two different neurotoxins were treated with different concentrations of sotalol. Cell survival (%) was calculated relative to control cells not exposed to any neurotoxin or to sotalol. The p value was calculated relative to cells exposed to the neurotoxin, but not treated with sotalol.

Fig. 11A: cells treated with Aβ peptide, as a neurotoxin and with 100μM of sotalol.

Fig. 11B: cells treated with 3-nitropropionic acid (3-NP), as a neurotoxin and with different concentrations (5-100μM) of sotalol.

Abbreviations: con. (control).

Figure 12A-12B: Flecainide shows neuroprotection in PC12 neuronal cell cultures against Aβ peptid (model AD) and against 3-NP (model of HD)

PC-12 cells exposed to two different neurotoxins were treated with different concentrations of flecainide. Cell survival (%) was calculated relative to control cells not exposed to any neurotoxin or to flecainide. The p value was calculated relative to cells exposed to the neurotoxin, but not treated with flecainide.

Fig. 12A: cells treated with Aβ peptide, as a neurotoxin and with 50-200μM of flecainide.

Fig. 12B: cells treated with 3-nitropropionic acid (3-NP), as a neurotoxin and with different concentrations (50-200μM) of flecainide. Abbreviations: con. (control).

Figure 13: Propafenone shows neuroprotection in PC12 neuronal cell cultures against Aβ peptide (model AD)

PC-12 cells exposed to Aβ peptide, were treated with different concentrations of propafenone (5-100μM). Cell survival (%) was calculated relative to control cells not exposed to the neurotoxin or to propafenone. The p value was calculated relative to cells exposed to the neurotoxin, but not treated with propafenone. Abbreviations: con. (control).

Figure 14A-14B: Effect of amiodarone on the clinical course of MOG-EAE and PLP-EAE in C57Bl and SJL mice, respectively

Fig. 14A: Mice were immunized with MOG to induce EAE and were either left untreated (N=16) or received 30mg/Kg/day of amiodarone i.p. (N=12), starting at the day of MOG immunization. Animals were examined for signs of EAE and scored according to a 0-6 point disability scale of the disease (0- no abnormality, 1- mild tail weakness, 2- tail paralysis, 3- tail paralysis and hind leg paresis, 4- hind leg paralysis or mild forelimb weakness, 5- quadriplegia or moribund state, 6- death). The figure shows combined results of 3 separate experiments, each showing these trends.

Fig. 14B: Mice were immunized with PLP to induce EAE and were either left untreated (N=5) or received 60mg/Kg/day of amiodarone i.p. (N=6), starting at day 7 of PLP immunization. Animals were examined for signs of EAE and scored according to a 0-6 point disability scale of the disease. Abbreviations: con. (control), trea. (treatment), D. (days), po. (post), imm. (immunization), mea. (mean), cli. (clinical), sco. (score).

Detailed Description of the Invention

The present inventors show in PC12 neuronal cell cultures, that amiodarone (an antiarrhythmic drug) exerts neuroprotection against Aβ peptide, and that ezetimibe (a cholesterol absorption inhibitor) exerts neuroprotecion against both toxic conditions, Aβ peptide and high cholesterol. Amiodarone further demonstrated neuroprotection against experimental autoimmune encephalomyelitis (EAE), an animal model for inflammation in the CNS, as well as against experimental PD and ALS, as shown in animal models. The present invention further discloses the neuroprotective potential of additional antiarrhythmic drugs such as sotalol, flecainide and propafenone. These drugs are currently in use for treatment of cardiac arrhythmias. The neuroprotective properties of all these known drugs were demonstrated in neuronal culture model, and some of them also in animal models.

Thus, in a first aspect, the invention relates to a method for the treatment or prevention of a pathologic disorder, in a subject in need thereof. The pathologic disorder treated by the invention may be selected from neurodegenerative disorders and autoimmune-related disorders. According to one embodiment, the method of the invention comprises the step of administering to a subject in need, a therapeutically effective amount of any one of an antiarrhythmic agent, a cholesterol absorption

inhibitor agent, a composition comprising the same or any combinations thereof.

The terms "treat, treating, treatment" as used herein and in the claims mean ameliorating one or more clinical indicia of disease activity in a patient having a neurodegenerative disease.

"Treatment" refers to therapeutic treatment. Those in need of treatment are mammalian subjects suffering from any neurodegenerative disorder or an autoimmune-related disorder. By "patient" or "subject in need" is meant any mammal for which administration of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, or any pharmaceutical composition comprising this compound or derivatives thereof is desired, in order to prevent, overcome or slow down such infliction.

To provide a "preventive treatment" or "prophylactic treatment" is acting in a protective manner, to defend against or prevent something, especially a condition or disease. It should be noted that the preventive effect of amiodarone, is clearly demonstrated in Example 3 and Figure 6. This Example shows that amiodarone ameliorated PD symptoms in a PD-animal model, when administered i.p. or by drinking water, starting two or four weeks before disease induction, respectively.

The method of the invention is based on the administration of a therapeutically effective amount of an antiarrhythmic agent, and/or a cholesterol absorption inhibitor agent. The terms "effective amount" or "sufficient amount" mean an amount necessary to achieve a selected result. The "effective treatment amount" is determined by the severity of the disease in conjunction with the preventive or therapeutic objectives, the route of administration and the patient's general condition (age, sex, weight and other considerations known to the attending physician).

The method of the invention should be applied to a subject suffering from a pathologic disorder such as a neurodegenerative disorder or an autoimmune-related disorder. As used herein, the term "disorder" refers to a condition in which there is a disturbance of normal functioning. A "disease" is any abnormal condition of the body or mind that causes discomfort, dysfunction, or distress to the person affected or those in contact with the person. Sometimes the term is used broadly to include injuries, disabilities, syndromes, symptoms, deviant behaviors, and atypical variations of structure and function, while in other contexts these may be considered distinguishable categories. It should be noted that the terms "disease", "disorder", "condition" and "illness", are equally used herein.

A "neurological disorder" is a disease or disorder characterized by an abnormality or malfunction of neuronal cells or neuronal support cells. The disorder can affect the central and/or peripheral nervous system. Exemplary neurological diseases include neuropathies, skeletal muscle atrophy and neurodegenerative diseases.

"Neurodegenerative disorders" are complex and pernicious diseases, their onset is insidious, followed by progressive deterioration. Clinical manifestations are determined by the location and seriousness of the disorder. Although the causes may differ, patients with neurodegenerative disorders are likely to show localized to generalized atrophy of brain cells, leading to compromises in both mental and physical function. Exemplary neurodegenerative diseases include: Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, taupathies such as Pick's disease, fronto temporal dementia, cortico-basal degeneration and progressive supranuclear palsy and Spongiform encephalopathies such as Scrapie, mad cow disease and Bovine

spongiform encephalopathy, Creutzfeldt-Jakob disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru.

Mentally, patients will exhibit forgetfulness, poor memory, decrease in mental capacities, emotional disturbances, and/or poor speech. Physically, patients will exhibit partial to complete incontinence, aspiration of food particles, tremor, poor balance, muscle rigidity, and/or muscle paralysis.

According to one specific embodiment, the antiarrhythmic agent as well as the cholesterol absorption inhibitor agent used by the method of the invention, exert neuroprotective effect and improve neuronal survival upon exposure to neurotoxic agents.

"Neurotoxic agents" are death-promoting factors to the nervous system cell constituents. Such neurotoxic agent may be for example, any one of cholesterol, preferably free cholesterol, Aβ peptide, in particular the peptide of SEQ ID No:1, alpha-synuclein, huntingtin, prion protein, 3-NP (3-nitropropionic acid), MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, 6OHDA (6-hydroxydopamine) and Glutamate.

A "neuroprotective effect" is aimed to prevent and treat complications that might result in central nervous system (CNS) damage. Neuroprotection can be estimated by parameters of cell survival or cell death delay, arrest or slowing of the disease progression, disease onset and disease mortality delay.

Neuroprotective agents usually interact with the cell survival/apoptotic machinery. Products with neuroprotective effects include those from the categories of free radical scavengers, anti-excitotoxic agents, apoptosis (programmed cell death) inhibitors, anti-inflammatory agents,

neurotrophic factors, metal ion chelators, ion channel modulators and gene therapy.

Vitamin E and vitamin C are known to exert protective effect against atherosclerosis and have been proposed to be neuroprotective agents in aging and neurodegenerative diseases.

Vitamin E (antioxidant) pretreatment of embryonic neurons cultured in the presence of neurotoxic oxidized-LDL (oxLDL) prevented neurons death [Sugawa M. et al., (1997) Brain Res. 761:165-172]. Similar protective effects were reported for vitamin E against oxLDL toxicity in cultured PC-12 cells [Draczynska-Lusiak B., Doung A. and Sun AY. (1998) Mol. Chem. Neuropathol. 33:139-148].

Neuroprotective therapies are usually directed to cerebrovascular disorders, traumatic brain injury, spinal cord injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis,, Huntington's disease, taupathies, multiple sclerosis, epilepsy and ischemic optic neuropathy.

It should be appreciated that any of the antiarrhythmic drugs and/or cholesterol absorption inhibitor compounds used by the method of the invention, may be combined with any of the known neuroprotective agents described for example, above.

In one particular embodiment, the invention relates to a method for treating neurodegenerative disorders selected from protein misfolding disorders, amyloid diseases, taupathies or prion diseases.

The terms "Amyloid disease", "Amyloid condition" or "Amyloidosis" refers to deposits of proteins causing diseases. Occasionally, cells produce abnormal proteins that can settle in body tissue, forming deposits and

causing disease. The deposits of abnormal proteins are called amyloids, and the disease process amyloidosis.

Examples of diseases that feature amyloid deposition and their associated proteins (indicated in parentheses) include Alzheimer's disease (aβ 1-42), Parkinson's disease (alpha-synuclein), Huntington's disease (huntingtin), Spongiform encephalopathies such as Creutzfeldt-Jakob disease (PrP in cerebrum), Kuru (diffuse PrP deposits in brain), Fatal Familial Insomnia (PrP in thalamus), scrapie and Bovine Spongiform Encephalopathy (PrP in cerebrum).

"Prion diseases" often called "spongiform encephalopathies", are a group of progressive conditions that affect the brain and nervous system of humans and animals. The disorders cause degenerative diseases of the nervous system reflected by impairment of brain function, including memory changes, personality changes, and problems with movement that worsen over time. Probably most mammalian species develop these diseases. The infectious agent causing the diseases has been called a prion. A "prion" has been defined as a small proteinaceous infectious particle which resists inactivation by procedures that modify nucleic acids. Prions are microscopic protein particles similar to a virus but lacking nucleic acid, capable of self-reproducing. Though their exact mechanisms of action and reproduction are still unknown, it is accepted that they are responsible for a number conditions in humans, Creutzfeld-Jacob Disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI), Kuru, Alpers Syndrome; and in cattle (livestock), Scrapie in sheep, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in mule deer or elk, and bovine spongiform encephalopathy (BSE) in cows.

Abundant cytoplasmic inclusions consisting of aggregated hyperphosphorylated protein tau, called neurofibrillary tangles (NFT), are a characteristic pathological observation in several neurodegenerative disorders, known as taupathies. These disorders include Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

In yet another specific embodiment, the method of the invention is intended for the treatment of an autoimmune disorder. Preferably, an autoimmune disorder related to the CNS. Most preferably, an autoimmune CNS inflammatory disorder, for example, multiple sclerosis and acute disseminating encephalomyelitis.

It should be appreciated that although the invention is particularly directed to inflammatory CNS-related disorders, other autoimmune disorders may be contemplated within the scope of the invention. Thus, in general, the method of the present invention may be used in the treatment of any autoimmune disease such as for example, but not limited to, Eaton-Lambert syndrome, Goodpasture's syndrome, Greave's disease, Guillain-Barr syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjogren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behget's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, insulin dependent diabetes, inflammatory bowel disease, ulcerative colitis and Crohn's disease.

According to a specifically preferred embodiment, the method of the invention is specifically suitable for the treatment of a mammalian subject. "Mammal" or "mammalian" for purposes of treatment refers to any animal classified as a mammal including, human, research animals, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. In a particular embodiment said mammalian subject is a human subject. In another preferred embodiment, said mammalian subject is a bovine subject, preferably a cow or a sheep.

In one preferred embodiment, the method of the invention comprises the step of administering to a subject in need thereof, a therapeutically effective amount of an antiarrhythmic agent or of a composition comprising the same.

Antiarrhythmic medications are, as the name suggests, a group of medications used to treat cardiac arrhythmias, which are any abnormalities in the heart's rate or rhythm. Antiarrhythmic medicines act by blocking adrenergic receptors and by binding to ion channels, such as sodium, potassium, and calcium channels.

The terms "adrenergic" or "adrenoreceptor" describe a nerve or other cell, or cell receptor (adrenoreceptor) that is activated by epinephrine, norepinephrine, or an epinephrine-like substance. Also describes a nerve which releases such substances from its nerve ending. "Noradrenergic" relates to an adrenergic nerve cell that by itself liberates noradrenaline (ie. norepinephrine) or to a cell or receptor that is stimulated preferentially by norepinephrine.

The adrenergic receptors, which subserve the responses of the sympathetic nervous system, are divided into two discrete subtypes: alpha adrenergic

receptors (alpha receptors subtypes α1, α2, α1A, α 1D, α2A and α2D) and beta adrenergic receptors (beta receptors subtypes β1, β2 and β3) based on the interaction of agonists and antagonists with the receptors.

Beta-blockers may be the most common antiarrhythmic medicines used by physicians. They block the nervous system from exciting the heart and tend to slow the heart rate slightly. These medicines are used for treating high blood pressure, coronary artery disease, and congestive heart failure in addition to arrhythmias. Part of the reason they are such helpful medicines for patients with coronary artery disease and congestive heart failure is that they help prevent cardiac arrhythmias.

Ion channels are integral membrane proteins. A hollow centre through the channel protein forms a water-filled pore through which ions permeate. Ion channels are referred to by the type of permeating ion. Potassium channels specifically allow potassium ions to cross the cell membrane through a water-filled channel protein. They are designed to allow the flow of potassium ions across the membrane, but to block the flow of other ions, in particular, sodium ions.

Calcium channel-blockers are medicines that bind to a calcium channel in the heart and blood vessels. Some calcium channel-blockers may slow the heart rate slightly; these medications are useful in treating arrhythmias. Sodium and potassium channel-blocking drugs have been in use for many years. They bind to proteins in the heart that allow either sodium or potassium to pass into and out of cells. There are many of these medications on the market and some even have "combined activities," meaning they bind to other channels in the heart as well. This group of medicines is most useful in stopping or preventing a variety of arrhythmias including atrial fibrillation, atrial flutter, supraventricular tachycardia (SVT) and ventricular arrhythmias.

Singh and Vaughan Williams [Singh B.N. and Vaughan Williams E.M. (1970) Br. J. Pharmacol. 39: 675–686] developed a standard classification of the antiarrhythmic drugs based upon the antiarrhythmic drug's electrophysiological mechanisms of action:

Class I drugs act by blocking the sodium channel and are subdivided into 3 subgroups, IA, IB, and IC based on their effects on repolarization and potency towards blocking the sodium channel. Subclass IA has high potency as sodium channel blockers and also usually prolongs repolarization through blockade of potassium channels; subclass IB drugs have the lowest potency as sodium channel blockers and shorten repolarization; subclass IC drugs are the most potent sodium channel blocking agents and have little effect on repolarization.

Class II drugs act indirectly on electrophysiological parameters by blocking beta-adrenergic receptors.

Class III drugs prolong repolarization (increase refractoriness) by blocking outward potassium conductance with typically little effect on the rate of depolarization.

Class IV drugs are relatively selective AV nodal L-type calcium-channel blockers.

Miscellaneous a miscellaneous group of drugs that includes digoxin, adenosine, magnesium, alinidine (a chloride channel blocker) and other compounds whose actions don't fit the standard four classes.

In one embodiment, the invention relates to a method in which the use of an antiarrhythmic agent exerts neuroprotective effect and improves neuronal survival upon exposure to neurotoxic agents.

The neuroprotective effect of amiodarone treatment on PC-12 cell survival exposed to Aβ (25-35) peptide (SEQ ID No:1) toxic conditions is detailed in

Example 2 and Figure 4. PC-12 cells treated with 25–800μM amiodarone, overcame the neurotoxic effect of the Aβ (25-35) peptide.

A significant higher cell survival can be observed in the treated cells as compared to control cells (amiodarone untreated cells). Cell survival in the amiodarone treated cells reached 100%, while only 26% of the cells survived the Aβ peptide toxicity in the control group (no amiodarone treatment) ($p= 2x10^{-5}$). The effect of 25-100μM amiodarone concentration treatment completely abolished the neurotoxic effect of the Aβ (25-35) peptide, showing similar cell viability to the cells that were not exposed to Aβ (25-35) at all.

Higher concentrations of amiodarone were less effective since smaller neuroprotection extent against the Aβ peptide was observed. PC-12 cell survival when treated with 200, 400 or 800μM amiodarone was 65.9% ($p=0.0005$), 44.5% ($p=0.013$) and 37.6% ($p=0.12$), respectively.

The neuroprotective effect of amiodarone was also demonstrated in animal model related to PD (Example 3), ALS (Example 4) and EAE (Example 8). The neuroprotective effect of other antiarrhythmic drugs such as sotalol, flecainide and propafenone is demonstrated by Examples 5, 6, and 7, respectively.

As previously remarked, neuroprotective agents usually interact with the cell survival/apoptotic machinery. Antiarrhythmic drugs acting as ion channel modulators might interfere with the apoptosis signaling pathways.

Nerve cells self-destroy when the brain or spinal cord is injured or becomes diseased. In the hours or days after the brain is injured, healthy cells surrounding the damaged region also die, killing themselves through

a genetically controlled process called programmed cell death or apoptosis. Scientists previously have focused attention on changes in the amount of calcium contained within cells undergoing apoptosis. Mass exodus of potassium may be just as important as changes in calcium [Yu S.P. et al., (1997) Science 278: 114-117].

Potassium ions cross the cell membrane through tunnels called potassium channels, which control the rate at which they flow. If these channels play a key role in apoptosis, the use of drugs that are potassium channel blockers minimize potassium loss and prevent nerve cells from dying. This might restrict damage to the initially injured area of the brain or spinal cord, allowing patients to recover more fully. Manipulating potassium channels may provide a new therapeutic strategy for slowing neuronal degeneration in neurodegenerative disorders such as Alzheimer's disease.

More particularly, the antiarrhythmic agent may be any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

The antiarrhythmic agent amiodarone is also called amiodarone HCl, 2-butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone, cordarone, L-3428, SKF-33134-A and methanone.

Flecainide acetate is benzamide, N-(2-piperidinylmethyl) -2, 5-bis (2, 2, 2-trifluoroethoxy)-monoacetate.

The chemical formula of Propafenone hydrochloride (RYTHMOL) is: 2'-[2-Hydroxy-3-(propylamino)-propoxyl-3-phenylpropriophenone hydrochloride, and its molecular formula is $C_{21}H_{27}NO_3$ HCL and its molecular weight is 377.92.

The chemical formula of sotalol hydrochloride is d, 1-N-[4-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]phenyl]methane-sulfonamide monohydrochloride. The molecular formula is $C_{12}H_{20}N_2O_3S$ HCL, and its molecular weight is 308.8.

In a broad embodiment, the antiarrhythmic agent of the invention is amiodarone or active derivatives thereof. Amiodarone is a powerful and effective antiarrhythmic drug, commonly used in cardiac arrhythmias treatment. Its chemical formula is $C_{25}H_{29}I_2NO_3$ with a molecular weight of 645.31.

The term "active derivatives" refers to compounds derived or obtained from another and containing essential elements of the parent substance capable of functioning or producing an intended action or effect.

Amiodarone is considered a "broad spectrum" antiarrhythmic medication, that is, it has multiple and complex effects on the electrical activity of the heart which is responsible for the heart's rhythm.

In addition to being an antiarrhythmic medication, amiodarone also causes blood vessels to dilate (enlarge). This effect can result in a drop in blood pressure. Because of this effect it also may be of benefit in patients with congestive heart failure. Amiodarone does it all, it binds to sodium, potassium, and calcium channels in addition to being a beta-blocker. It is one of the most powerful antiarrhythmic medicines in suppressing arrhythmias. Benzofurane derivative is capable of blocking both α- and β-adrenoreceptors. Amiodarone is mainly a potassium channel blocker that prolongs the action potential duration and refractory period of all cardiac fibers. In the Vaughan Williams classification of antiarrhythmics, amiodarone is considered to be a predominantly class III agent, with some

class I properties, sotalol is considered as class III agent, flecainide as class IC, and propafenone as class IC agent.

In the method of the present invention, the therapeutically effective amount of amiodarone or any of the antiarrhytmic drugs used, that is sufficient to show a meaningful patient benefit should be determined by factors such as the age, weight and sex of the patient and mode of administration. Other considerations, for instance, the degree of amyloid plaque formation inhibition desired and the potency of amiodarone for the particular disorder of disease concerned should be cared about.

Preferred doses in humans are: (a) for oral treatment from about 100 to about 2000 mg per day taken in divided doses in acute episodes or beginning of the treatment and from about 10 (particularly in children) to about 500, preferably, 400 mg per day as a maintenance dosage. (b) for intravenous injection in acute cases, 100 to about 1000 mg per day are recommended for no longer than three weeks.

According to another specific embodiment, the active ingredient, an antiarrhythmic agent or any active derivatives thereof may be administered alone, or in combination with other active ingredients that improve the therapeutic effect, whether administered in combination, serially or simultaneously. As indicated herein before, the other ingredient may be any of the known neuroprotective agents.

In yet another specific embodiment, the other active ingredient may be a cholesterol absorption inhibitor agent, preferably, ezetimibe.

In another alternative and preferred embodiment, the method of the invention comprises the step of administering to a subject in need thereof, a therapeutically effective amount of a cholesterol absorption inhibitor

agent or of a composition comprising the same.

In a broad embodiment, the cholesterol absorption inhibitor agent of the invention is ezetimibe or active derivatives thereof.

The chemical name of ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone, ($C_{24}H_{21}F_2NO_3$) and its molecular weight is 409.4. The cholesterol absorption inhibitor agent ezetimibe, also commercially called "Ezetrol" and "Zetia", is a white, crystalline powder that is freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water.

The neuroprotective effect of ezetimibe treatment on PC-12 cell survival exposed to Aβ (25-35) peptide (SEQ ID No:1) toxic conditions is detailed in Example 1 and Figure 2. PC-12 cells treated with 25–100μM ezetimibe, overcame the neurotoxic effect of Aβ (25-35). A significant higher cell survival can be observed in the treated cells as compared to Aβ (25-35) treated cells, that were not treated with ezetimibe.

The ezetimibe neuroprotective effect is dose dependent, ranging from 64% ($p=0.1$) cell survival (treatment with 2.5μM ezetimibe) up to 107% ($p=0.01$) survival (treatment with 100μM). Ezetimibe untreated cells showed only 45% cell survival. The effect of 50-100μM ezetimibe concentration treatment completely abolished the neurotoxic effect of the Aβ (25-35) peptide, showing similar cell viability to the cells that were not exposed to Aβ (25-35) at all.

The neuroprotective effect of ezetimibe, evaluated by cell survival (viability) after being exposed to high-cholesterol level neurotoxicity, is illustrated in Example 1 and Figure 3. Only 25% survival was observed in PC-12 cells incubated with 5μM cholesterol. Treatment with 100μM

ezetimibe significantly increased cell survival to 42% (p=0.04), indicating that ezetimibe exerts a protective effect in neuronal cells exposed to high-cholesterol induced neurotoxicity.

Excess levels of LDL and particularly the oxidized lipoproteins (e.g. ox-LDL and ox-HDL) are known to have cytotoxic effects on various neuronal cell cultures. Sugawa described the deadly effect of ox-LDL on cultured rat embryonic cerebral cortical neurons and human SH-SY5Y neuroblastoma cells [Sugawa M. et al., (1997) Brain Res. 761:165-172].

Co-application of oxLDL with either glutamate or Aβ peptide (two agents that enhance oxidative stress) results in a synergistic effect with increase in neuronal death [Keller JN., et al., (1999) J. Neurochem. 72:2601-2609].

Therefore, oxysterols and/or another component of oxLDL are considered active neurotoxic molecules.

The LDL-C reduction and safety profile of subjects already treated with ezetimibe are comparable between elderly (≥ 65 years) and young (18 to 45 years) as well as for men and women. Therefore, no treatment dosage adjustment is necessary on the basis of gender or the age (elderly).

In the method of the present invention, the term "therapeutically effective amount" means the total amount of ezetimibe that is sufficient to show a meaningful patient benefit, i.e., improve one or more clinical parameters of disease activity, e.g. retention or cognition, or improve disease symptoms such as anxiety or neuromotor control.

The dosage of ezetimibe needed to achieve a therapeutic effect will depend not only on such factors as the age, weight and sex of the patient and

mode of administration, but also on the degree of amyloid plaque formation inhibition desired and the potency of the particular compound being utilized for the particular disorder of disease concerned. Preferred ezetimibe doses in humans are from about 1 to 50 mg orally per day, preferably from about 5 to about 25 mg orally per day, and most preferably about 10 mg orally per day.

In the case of amyloid disorders, the subject amount is further characterized by its ability to inhibit or reduce plaque formation, as determined using *in vitro* assays or *in vivo* animal models of disease. For example in the case of Alzheimer's disease, the Aβ peptide accumulation or Aβ peptide induced cell death may be tested.

Experimental assays may include *in vitro* and *in vivo* experiments, based on the use of animal models, as presented in the invention.

The *in vitro* experimental model used in the present examples involves the use of PC-12 cell line. The PC-12 cell line is derived from a transplantable rat pheochromocytoma. The cells respond reversibly to NGF by induction of the neuronal phenotype. PC-12 cells are an immortal cell line derived from the cancerous tissue of a rat adrenal gland, but differ from other cancerous cells in that they have some very special properties. In their "normal" undifferentiated state, PC-12 cells exhibit the properties of a non-neuronal cell. However, if these cells are exposed to specific growth factors (e.g. NGF), they begin to change. Over the course of one week, the cells stop dividing, and they differentiate into a neuronal-like cell type. Therefore, these cells are used as excellent models for understanding the physiology of real neurons.

Suitable animal models for Alzheimer's disease investigation include, but are not restricted to the following examples:

(a) Mice animal models as described [Horikoshi Y. et al., (2004) Biochem. Biophys. Res. Commun. 325: 384-387; Costa DA. et al., (2004) J. Alzheimer's Dis. 6: 509-514; Yao Y. et al., (2004) J. Neuroinflammation 1: 21]. Such mice species might be transgenic strains, transgene congenic strains, targeted mutated strains or targeted mutated congenic strains. Mice strains in which the normal expression of any of the following genes is affected, might represent a suitable model for Alzheimer's diseases: APP (amyloid precursor protein), apoE (Apolipoprotein E), α synuclein, COX-2 (Cyclooxygenase-2), PS1 (presenilin-1), PS2 (presenilin-2) or Tau (microtubule associated protein Tau).
(b) Exemplary rat models as described [Ricceri L. et al., (2004) Exp. Neurol. 189: 162-172; Grunblatt E., et al., (2004) J. Neural Transm. 111: 367-86].
(c) Monkey models [Lemere CA. et al., (2004) Am. J. Pathol. 165: 283-297; Gandy S. et al., (2004) Alzheimer Dis. Assoc. Disord. 18:44-46] are also known.

The neuroprotective effect of ezetimibe in an AD animal model is under investigation. Animals treated with ezetimibe are tested for the prevention of the development of the senile amyloid plaques and for the amyloid burden reduction in already affected animals and in animals presenting neurofibrillary tangles.

Delayed onset or reduced burden in the AD animal model's brain pathology, are considered a treatment success and ezetimibe should be considered in clinical studies for the prevention and treatment of AD in patients.

According to one embodiment, the active ingredient, preferably, ezetimibe may be administered alone, or in combination with other active ingredient/s that improve the therapeutic effect, whether administered in

combination, serially or simultaneously.

Accordingly, the other active ingredient may be an antiarrhythmic agent, for example, any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

According to a preferred embodiment, the method of the invention includes any administration such as oral, intravenous, intramuscular, subcutaneous, intraperitoneal, parenteral, transdermal, intravaginal, intranasal, mucosal, sublingual, topical, rectal or subcutaneous administration, or any combination thereof.

It should be appreciated that the present invention represent different methods of administration including i.p. injections, oral gavage and oral administering by drinking water (Example 3, 4 and 8).

It is also contemplated that the treatment and dosage of the particular compound may be administered in unit dosage form and that the unit dosage form would be adjusted accordingly by one skilled in the art to reflect the relative level of activity. The decision as to the particular dosage to be employed (and the number of times to be administered per day) is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired therapeutic effect.

In a second aspect, the invention relates to the use of an antiarrhythmic agent and/or a cholesterol absorption inhibitor agent in the preparation of a pharmaceutical composition for the treatment or prevention of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune related disorder, preferably, a CNS inflammation disorder, in a subject in need thereof.

According to one embodiment, the pathologic disorder may be a neurodegenerative disorder selected from protein misfolding disorders, amyloid diseases, taupathies or a prion disease.

More specifically, a neurodegenerative disorder may be for example, any one of Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, taupathies such as Pick's disease, fronto temporal dementia, cortico-basal degeneration and progressive supranuclear palsy and Spongiform encephalopathies such as Scrapie, mad cow disease and Bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru.

It should be noted that any one of an antiarrhythmic agent and a cholesterol absorption inhibitor agent used by the invention, exert neuroprotective effect and improves neuronal survival upon exposure to neurotoxic agents.

These neurotoxic agents may be for example, cholesterol, preferably free cholesterol, Aβ peptide, in particular the peptide of SEQ ID No:1, alpha-synuclein, huntingtin, prion protein, 3-NP (3-nitropropionic acid), MPTP (1-methyl-4-phenyl- 1, 2, 3, 6-tetrahydropyridine, 6OHDA (6-hydroxydopamine) and Glutamate.

According to another specific embodiment, an antiarrhythmic agent and/or a cholesterol absorption inhibitor agent may be used for the preparation of a pharmaceutical composition for the treatment of an autoimmune disorder such as, multiple sclerosis and acute disseminating encephalomyelitis.

It should be appreciated that the pharmaceutical composition prepared by the invention is particularly suitable for the treatment of a mammalian subject.

According to one particular and preferred embodiment, the present invention relates to the use of an antiarrhythmic agent in the preparation of pharmaceutical composition for the treatment or prevention of a pathologic disorder such as a neurodegenerative disorder or an autoimmune-related disorder, in a subject in need thereof.

More specifically, the antiarrhythmic agent used by the invention may be any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof. Preferably, the antiarrhythmic agent is amiodarone or any physiologically acceptable active derivatives thereof.

According to another particular and preferred embodiment, the present invention relates to the use of a cholesterol absorption inhibitor agent in the preparation of pharmaceutical composition for the treatment or prevention of a pathologic disorder such as a neurodegenerative disorder or an autoimmune related disorder, in a subject in need thereof.

In a specifically preferred embodiment, the cholesterol absorption inhibitor agent is ezetimibe.

This pharmaceutical composition may further comprise an additional active agent selected from cholesterol lowering agents, growth factor, hormones, analgesics, antibiotics, anti-inflammatory agents, anti-oxidants, and cyto-protectants, especially neuroprotectants.

The preparation of pharmaceutical compositions is well known in the art and has been described in many articles and textbooks, see e.g., Remington's Pharmaceutical Sciences, Gennaro A. R. ed., Mack Publishing Co., Easton, PA, 1990, and especially pp. 1521-1712 therein, fully incorporated herein by reference.

The pharmaceutical composition of the invention can be administered and dosed in accordance with good medical practice. Administration may be carried out in various ways, including intravenous, intraperitoneal, intramuscular or subcutaneous injection. However, other methods of administration such as nasal or oral administration may be preferred.

The composition of the invention may comprise the active substance in free form and be administered directly to the subject to be treated. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient.

Formulations include those suitable for oral, nasal, or parenteral (including subcutaneous (s.c.), intramuscular (i.m.), intraperitoneal (i.p.), intravenous (i.v.) and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The nature, availability

and sources, and the administration of all such compounds including the effective amounts necessary to produce desirable effects in a subject are well known in the art and need not be further described herein.

The pharmaceutical forms suitable for injection use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringe ability exists. The compositions must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption.

In the case of sterile powders for the preparation of the sterile injectable solutions, the preferred method of preparation are vacuum-drying and freeze drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The pharmaceutical compositions of the invention generally comprise a buffering agent, an agent that adjusts the osmolarity thereof, and optionally, one or more pharmaceutically acceptable carriers, excipients and/or additives as known in the art. Supplementary active ingredients can also be incorporated into the compositions. The carrier can be solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the

like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic composition is contemplated.

In a third aspect, the present invention relates to a method for protection of neuronal cells from a neurodegenerative process. The method of the invention comprises the step of contacting these neuronal cells with a neuroprotective effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof.

According to one embodiment, the neurodegenerative process may be caused by a neurodegenerative disorder in a mammalian subject. For example, a protein misfolding disorder, an amyloid disease, taupathy or a prion disease.

More particularly, the neurodegenerative disorder may be any one of Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, taupathies such as Pick's disease, fronto temporal dementia, cortico-basal degeneration and progressive supranuclear palsy and Spongiform encephalopathies such as Scrapie, mad cow disease and Bovine spongiform encephalopathy, Creutzfeldt-

Jakob disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru.

Alternatively, the neurodegenerative process to be prevented by the method of the invention may be caused by exposure the neuronal cells to a neurotoxic agent.

For example, the neurotoxic agent may be any one of cholesterol, preferably free cholesterol, Aβ peptide, in particular the peptide of SEQ ID No:1, alpha-synuclein, huntingtin, prion protein, 3-NP (3-nitropropionic acid), MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, 6OHDA (6-hydroxydopamine) and Glutamate.

According to a specifically preferred embodiment, the method of the invention comprises the step of contacting neuronal cells undergoing a neurodegenerative process, with a neuroprotective effective amount of an antiarrhythmic agent or of a composition comprising the same. Suitable antiarrhythmic agent may be amiodarone, sotalol, flecainide, propafenone or any physiologically acceptable active derivatives thereof. Preferably, amiodarone or any active derivatives thereof.

In yet another specific embodiment, the invention relates to a method for protection of neuronal cells from a neurodegenerative process, comprising the step of contacting said cells with a neuroprotective effective amount of a cholesterol absorption inhibitor agent, preferably, ezetimibe, or of a composition comprising the same.

The invention further provides for an antiarrhythmic agent, preferably, being any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof, for use in the treatment of neurodegenerative disorders.

Still further, the invention provides a cholesterol absorption inhibitor agent, preferably, ezetimibe, for use in the treatment of neurodegenerative disorders.

The present invention further provides a method of preparing a therapeutic composition for the treatment of a pathologic disorder such as, a neurodegenerative disorder or an autoimmune related disorder, in a mammalian subject. This method comprises the steps of: (a) providing any one of an antiarrhythmic agent and a cholesterol absorption inhibitor agent; and (b) admixing the antiarrhythmic agent, the cholesterol absorption inhibitor agent or any combination thereof, with at least one of a pharmaceutically acceptable carrier, diluent, excipient and/or additive.

Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The following Examples are representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications can be made without departing from the inventive concepts.

The invention will be described in more detail on hand of the following examples which are illustrative and do not limit its scope, which is only defined by the appended claims.

Examples

Experimental procedures

Tested compounds

Ezetimibe *(Ezetrol, MSD Schering Plough);*

Amiodarone (Amiodacore, C.T.S.; and Procor, Unipharm);

Sotalol (Sotalol, Genmedix);

Flecainide (Tambacor , Pharmateam Marketing);

Propafenone (Rythmex, Teva);

Neurotoxins

*Aβ (25-35) peptide was purchased from Sigma.

*Cholesterol purchased from Sigma.

*3-NP purchased from Sigma.

*MPTP purchased from Sigma.

*6OHDA purchased from Sigma.

*Glutamate purchased from Sigma.

Animals

*Male Sprague-Dawley rats (weighing 250-270g), purchased from Harlan Laboratories Israel, were used as a PD model.

*14-15 weeks old transgenic mice expressing the human G93A SOD1 (B6SJL-TgN[SOD1-G93A]1Gur, purchased from Jackson Laboratory), were used as a model showing ALS related clinical symptoms.

*Transgenic mice model expressing a mutation in the amyloid precursor protein (APP) and in the presenilin1 gene, was used as AD model. These tg mice present amyloid plaques starting at 9 months of age (purchased from Jackson Laboratory).

*Tg mice model expressing double mutants in the tau protein, was used as AD model. These tg mice present neurofibrillary tangles starting at 7 months of age. These mice were generated by the inventors [strain background CB6F1 (C57Bl XBalbC)].

*C57Bl mice, purchased from Harlan Laboratories Israel, immunized with MOG.

*SJL/j mice immunized with PLP, were purchased from Harlan Laboratories Israel.

PC-12 cell culture

PC-12 cells were grown in Dulbeco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum, 10% horse serum, 100μg/ml streptomycin, and 100U/ml penicillin. The cultures were maintained in an incubator at 37°C in a humidified atmosphere of 5% CO_2. $5x10^4$ cells/ml were cultured in 24-well plates. Cells were subjected to neurotoxins and neuroprotection tests as described below.

Treatment of PC-12 with neurotoxins

*For Aβ toxicity, Aβ (25-35) peptide (1-2μM final concentration) was incubated for 10 minutes at 37°C before added to cells.

*For cholesterol toxicity 5μM cholesterol were added to the medium.
*Serum-free cytotoxicity was tested growing the cells in the medium without serum supplement.

The following neurotoxin treatment procedures were applied for Figures 1-5, 11-13:

Following two hours of neuroprotection treatment (or concomitant with the neuroprotection treatment) the following neurotoxins were added separately to PC-12 cells, and incubated for 16-20 hours at 37°C: 5μM Aß (25-35) peptide, 5μM cholesterol ,10mM 3-NP, 5mM MPTP, 200μM 6OHDA, or 25mM glutamate.

Neuroprotection of PC-12 cells

*Neuroprotection against the Aβ (25-35) peptide was presented with 1μM simvastatin and with 100μM vitamin E together with 100μM vitamin C.
*In the ezetimibe treatments, ezetimibe at final concentrations of 2.5-100μM was added to cells incubated with the Aβ (25-35) peptide, as shown by Figures 1-2.
*For experiments shown in Figures 4-5,11-13, the following potential neuroprotective compounds (5-200μM) were incubated with the cells for two hours: amiodarone, sotalol, flecainide and propafenone. This was followed by exposure the cells to various neurotoxins, which mimic various neurodegenerative conditions.

In all experiments cell viability was tested following 24 hours of incubation.

Cell viability

The cell viability assay was based on the ability of cellular mitochondria to convert MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium

bromide) into a blue formazan product and was performed similar to the procedure described by others [Miller R, CA. (1991) Anal. Biochem. 192: 380-383, and Heo H. et al., (2004) J. Agric. Food Chem. 52: 4128-32]. Briefly, the cells were incubated with 1mg MTT/ml for 2 hours at 37°C. The medium containing MTT was discharged, and the attached cells were dissolved in 150µl of dimethylsulfoxide. The amount of MTT formazane product was determined by measuring the absorbance using a microplate reader at 550nm. Percentage of cell survival was calculated relative to untreated control cells.

Statistical analysis

For comparison of % cell survival in the experiments the unpaired student t-test analysis was used. In the neurotoxic conditions p value was calculated relative to the untreated control cells. In the neuroprotective conditions p value was calculated relative to the cells exposed to the toxic conditions (like Aβ peptide, cholesterol or the others) without any neuroprotective treatment.

Studies in animal models

Model for PD

The neurotoxin 6OHDA (8µM /rat) was stereotaxically injected in 4µl into the right substantia nigra of male Sprague-Dawley rats (weighing 250-270g), for induction of nigral lession. This animal model show PD-related clinical symptoms within 3 days following 6OHDA injection.

*Amiodarone treatment

Rats were treated with amiodarone (ampule of Amiodacore-HCl in benzyl alcohol) in the following paradigms:

1. 30-40mg/Kg amiodarone in drinking water, starting 4 weeks before 6OHDA injection (N=6), versus water drinking controls (N=6). At day 50 post 6OHDA injection, treatment was stopped.

2. Daily i.p. of 20mg/Kg diluted in 5% dextrose, starting 2 weeks before 6OHDA injection (N=8; 2 animals developed distended abdomen and died), versus 5% dextrose injected controls (N=6). Animals were sacrificed at day 50. Striata were kept for HPLC analysis of the presence of dopamines.
3. Daily i.p. 20mg/Kg diluted in 5% dextrose, starting at day of 6OHDA injection (N=8; 5 developed distended abdomen and died), versus 5% dextrose injected controls (N=6).
4. Daily gavage of 75-100mg/Kg, starting at day of 6OHDA injection (N=5), versus controls (N=4). Animals were sacrificed at day 30. Striata were kept for HPLC analysis of presence of dopamines.
5. Daily i.p. 50mg/Kg diluted in 5% dextrose, starting 70 days after of 6OHDA injection (N=3), versus 5% dextrose injected controls (N=3).

**Stepping adjustments test*

The number of stepping adjustments was counted for each forelimb during slow sideway movements in forehand and backhand directions over a standard flat surface. The stepping adjustments test was repeated three times for each forelimb.

**Forelimb-placing test*

The forelimb-placing test assesses the rats' ability to make directed forelimb movements in response to a sensory stimulus. Rats were held with their limbs hanging unsupported. They were then raised to the side of a table so that their whiskers made contact with the top surface while the length of their body paralleled the edge of the tabletop. Control rats place their forelimb on the tabletop in response to whisker stimulation almost every time, whereas injured rats do not. Each test included 10 trials of placement of each forelimb and was repeated daily for three consecutive days. The results of both tests are expressed as percentage of forelimb stepping adjustments and placing in the lesioned side, as compared with the nonlesioned side. ANOVA analysis of repeated

measures was used to determine significant differences in the motor performance.

*At the end of experiment striata of the animals were collected, and submitted to HPLC analysis for the presence of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). The levels of dopamine and metabolites in the lesioned side of the treated animals (relative to the nonlesioned side) were compared to the non-treated animals.

In addition, in some of the animal's brain sections were submitted to immunohistochemistry analysis for tyrosine hydroxylase (TH), a rate-limiting enzyme for dopamine synthesis, which serves as an important marker of dopaminergic cell loss.

<u>Model for ALS</u>

Transgenic mice expressing the human G93A SOD1 (B6SJL-TgN[SOD1-G93A]1Gur), were used. These tg mice model show ALS related clinical symptoms starting at about 14 -15 weeks of age, and die at about 18-20 weeks.

Mice were treated with 30-40mg/Kg amiodarone (ampule of Amiodacore-HCl in benzyl alcohol, C.T.S Ltd.) in drinking water, starting at week 8 of age (N= 7), versus non-treated mice as controls (N=6).

**"Rotarod" motor function test*

Mice were weekly tested for motor function using a "Rotarod" device (Panlab, Barcelona) to detect onset and progression of disease-related weakness. The mice were placed on a rotating cylinder with a constant rate of acceleration. When the mice could no longer continue running and fall from the cylinder, the total time they spend on the cylinder and final speed achieved was recorded electronically, thus allowing calculation of

the total distance run. Each mouse performed three rotarod trials, and the best performance each week was recorded. A "baseline" distance was established at 12 weeks of age to which subsequent performance was compared. Onset of disease-related weakness was defined as a sustained decrease of more than 30% of baseline maximum running distance. Survival was defined by an accepted artificial endpoint as the time at which the mouse is no longer able to right itself within 30 seconds of being placed on its side).

A clinical 5-point score was used for assessing the ability to move:

4 = normal mobility; 3 = movement with limited use of the hindlegs; 2 = movement with the use of the forelegs; 1 = movement only for a short period with the use of the forelegs; and 0 = unable to move.

The time to onset and death/survival of treated and control groups were compared using Kaplen-Meier curve analysis (using SPS 12 for Windows ®). ANOVA analysis of repeated measures was used to determine significant differences in the motor performance.

Example 1

Neuroprotective effect of cholesterol absorption inhibitor

In order to establish a reliable cell culture system for screening the neuroprotective effect of different potential compounds, the neurotoxic effect of Aβ (25-35), cholesterol and serum-free conditions was first examined in PC-12 cell model. As shown in Figure 1, viability test of PC-12 cells clearly indicate neurotoxicity in exposure to 1μM Aβ (25-35), 5μM cholesterol and also under serum-free conditions (Fig. 1A). Treatment of these cells with known neuroprotective agents such as simvastatin (1μM), as well as vitamins E and C (100μM), that have been reported for their neuroprotective properties in epidemiological studies and even in clinical trails, resulted in neuroprotection as reflected by increase of cell viability

(Fig. 1B). It should be noted that vitamin E and vitamin C are known to exert protective effects against atherosclerosis and have been proposed to be neuroprotective agents in aging and neurodegenerative diseases.

The capability of the PC-12 cells to exhibit neurotoxic as well as neuroprotective effects makes these cells an authentic model for studying and screening for new neuroprotective drugs.

The inventors have thus demonstrated the neuroprotective effect of the cholesterol absorption inhibitor ezetimibe (Ezetrol) against the toxicity of the AD-related Aβ peptide. The effect is dose dependent at concentrations of 2.5-100μM, abolishing the cytotoxic effect of Aβ at 50-100μM ezetimibe.

Ezetimibe as neuroprotective agent

The effect of ezetimibe treatment on PC-12 cell survival exposed to Aβ toxic conditions was tested. PC-12 cells were treated with different concentrations ranging between 2.5–100μM ezetimibe. As shown by Figure 2, the treated cells were successfully rescued from the Aβ (25-35) neurotoxic effect. A significant higher cell survival is observed in the treated cells as compared to the ezetimibe untreated control cells. A dose response of the ezetimibe neuroprotective effect was noticed: treatment with 2.5μM ezetimibe resulted in 64% ($p=0.1$) cell survival, treatment with 5μM in 66% ($p=0.005$), treatment with 10μM in 78% ($p=0.03$), treatment with 25μM in 69% ($p=0.003$), treatment with 50μM in 97% ($p= 0.01$) and treatment with 100μM resulted in 107% ($p=0.01$) survival, relative to the 45% cell survival of the ezetimibe untreated cells.

The 50-100μM ezetimibe concentration completely abolished the neurotoxic effect of the Aβ (25-35) peptide, showing similar cell viability to cells that were not exposed to Aβ (25-35) at all. These results are based on

triplicates and similar trends have been observed in repeated experiments.

Neuroprotective effect of ezetimibe is mediated through its known function as a cholesterol absorption inhibitor

In order to test the possibility that the neuroprotective effect of ezetimibe is mediated through its known function as a cholesterol absorption inhibitor, the effect of ezetimibe on cell viability of PC-12 cells incubated with 5μM cholesterol demonstrated a decrease in cell viability to 25% (Figure 3). Treatment with 100μM ezetimibe significantly increased cell survival to 42% (p=0.04), indicating that ezetimibe exerts a protective effect in neuronal cells exposed to high-cholesterol induced neurotoxicity

Without being bound by any theory, it may be proposed that since amyloid toxicity is mediated by cholesterol levels [Mizuno T. et al., (1999) J. Biol. Chem. 274:15110-15114], the protective effect of ezetimibe against Aβ is probably mediated by its activity as a cholesterol absorption inhibitor. The neuroprotective effect of ezetimibe against high-cholesterol induced toxicity in the PC-12 cells also supports this concept.

Example 2

Amiodarone exhibits neuroprotection against Aβ peptide (model of AD), 3-NP (model of HD), MPTP and 6-OHDA (models of PD), and glutamate (model of ALS) in PC12 neuronal cell cultures

The effect of the antiarrhythmic drug amiodarone against the neurotoxic condition induced by the AD related Aβ peptide in PC-12 neuronal cell culture was investigated. The efficacy of the amiodarone treatment was estimated by assessing the percentage of PC-12 cell survival after being exposed to the Aβ (25-35) peptide toxic conditions.

Treatment of PC-12 cells with amiodarone at concentrations of 25-800μM, rescued the cells from the peptide neurotoxicity (Figure 4). At 25-100μM amiodarone treatment concentrations, the toxic effect of the Aβ (25-35) peptide on cell viability was abolished. Cell survival in the amiodarone treated cells reached 100%, while only 26% of the cells survived the Aβ peptide toxicity in the control group (no amiodarone treatment) ($p= 2x10^{-5}$). In other words, amiodarone reduces neuronal cell death caused by the Aβ peptide.

Higher concentrations of amiodarone seem less effective as reflected by the smaller neuroprotection extent against the Aβ peptide. PC-12 cell survival treated with 200, 400 or 800μM amiodarone was 65.9% (p=0.0005), 44.5% (p=0.013) and 37.6% (p=0.12) respectively, relative to untreated cells.

These results clearly point to a neuroprotective effect of amiodarone in neuronal-cell model, encouraging its use in the prevention and treatment of AD, and possibly other neurodegenerative disorders.

In order to assess the potential neuroprotective effect of amidorone on other neurodegenerative disorders, the rescue of PC-12 cells treated with different neurotoxins (presenting different neurodegenerative disorders) was next tested by the inventors. As shown in Figure 5, amiodarone at concentrations of 8-32μM protected PC12 cells against PD-related 6OHDA neurotoxicity. While the cell viability was reduced to 30% in the presence of 6OHDA, the treatment with 8, 16 and 32μM amiodarone rescued the cells, as shown by the significant increase in the cell viability to 55%(p=0.004), 55% (p=0.005), 51% (p=0.02), respectively (Fig. 5A). Similarly, neuroprotective effects were noticed when using the MPTP, another PD-related toxin. Cell viability was significantly increased from 14% (without treatment) to 20- 66% under the treatment with amiodarone

(p=0.004) (Fig. 5B). A neuroprotective effect of amiodarone was also detected under HD-related 3-NP toxicity (Fig. 5C), and under glutamate toxicity (Fig. 5D), when cell viability was significantly increased from 26 to 68% (p= 0.03), and from 40% to 95% (p= 0.002), respectively.

Example 3

Amiodarone ameliorated PD symptoms in a PD-animal model

The inventors next evaluated the neuroprotective effect of amiodarone on Parkinson's disease using an animal model. Rats stereotaxically injected with 6OHDA into the right substantia nigra developed PD-related motor deficits starting 3 days following injection. As demonstrated by the following experiments, rats treated with amiodarone showed amelioration of the motor deficits in the stepping and placing tests. The improvement in the symptoms was noticed in all the protocols that were tested: pre-treatment, early (day of disease induction) and late (day 70 post induction) treatments. Moreover, all modes of delivery showed effectiveness: drinking water, gavage, and i.p. injection. As shown by Figure 6, when amiodarone was given in drinking water starting 4 weeks of pre-treatment before disease induction, a significant better motor performance in stepping (Fig. 6A) and in placing (Fig. 6B) tests was detected, (p=0.0001 and p=0.01, respectively). The better performance was noticed at day 7 post induction (30% in stepping test , and 8% in placing test, compared to 0-2% performance in the untreated animals), reaching 43% and 13%, respectively, at day 42 (Figs. 6A, 6B). At day 50, when the amiodarone treatment was stopped, the performance reduced dramatically to 8-13% and to 0% in the stepping and placing tests, respectively (Figure 7). All the measures of motor performance in the lesions side were calculated relative to the non-lesioned side. Pre-treatment with amiodarone administered by i.p injections starting two weeks before disease induction, led to a significantly better motor

performance relative to non-treated controls, reaching 56%, and 23% in stepping and placing respectively (p=0.00009, p=0.004), compared to 0-2% performance in the untreated animals (Figs. 6C and 6D). Two animals out of 6 on the amiodarone treated group developed severe distended abdomen, and died during the experiment (this might be a result of the i.p. daily injections of amiodarone, which possibly caused peritonitis, a phenomenon which may be deduced from the pneumonitis reported as a side effect in patients treated with amiodarone for cardiac antiarrytmia [Lim K. and D. Radford (2002) Heart Lung Circ. 11: 59-62]). Testing the levels of dopamine in striatum of an amiodarone-treated rat, revealed the presence of 557ng/g tissue dopamine and 177ng/g tissue DOPAC in the lesioned side, versus 0 in a non-treated rat. These results indicate a possible involvement of amiodarone in dopamine synthesis.

The inventors next examined the neuroprotective effect of amiodarone, when administered with the neurotoxin. As shown by Figure 8, treatment with amiodarone at day of disease induction by i.p. injections, improved significantly the performance to 50% and 30% (at day 21) in stepping and placing, respectively (Fig. 8A and 8B) (p=0.000005, p=0.00001), compared to 0% performance in the untreated animals. It should be noted that also in this experiment, as in the previous i.p injected amiodarone animals, severe distended abdomen was developed, finally causing death (in 5 animals out of 8).

Treatment with amiodarone at day of disease induction by oral gavage improved significantly the performance to 40% in stepping test (p=0.001), compared to 0% performance in the untreated animals (Fig. 8C).

The inventors next checked the possible effect of amiodarone when i.p. administered 70 days post PD induction. As shown by Figure 9, treatment with amiodarone by i.p. injections at day 70 post disease induction

improved notably the performance to 17% in the stepping test, compared to 0% performance in the untreated animals. This difference did not reach a statistical significance, possibly because of the small number on animals tested (3 rats per group).

Example 4

Amiodarone delayed ALS onset and death in an ALS-animal model

The neuroprotective effect of amiodarone on AD and PD, encouraged the inventors to evaluate the feasibility of using this drug for the treatment of another neurodegenerative disease, ALS. As shown by Figure 10, ALS tg mice (N=7) treated with amiodarone (starting at 8 weeks of age) developed the disease significantly latter that the control ALS mice (N=6), as measured by reduced performance in the rotarod, (median value: onset at 14.5 and at 16.5 weeks of age, respectively, p=0.0009). A similar protective trend was noticed, when the disease development was evaluated using the clinical score. As shown by Figure 10B, a significant difference in onset of motor deficits was detected (median value: onset at 121 and at 130 days of age, in treated versus controls, respectively, p=0.03). Examining the survival of the animals the inventors detected a longer survival in the treated animals (median value: 139 versus 129 in treated versus controls, respectively, p=0.07). The potential beneficial effect of amiodarone in ALS mice was further investigated using different amounts of this compound. Results indicate that treatment of ALS tg mice with a 2 times higher level of amiodarone, did not demonstrate a protective effect. These results may indicate that monitoring the exact effective concentration for use may be necessary.

Example 5

Sotalol showed neuroprotection against Aβ (model of AD), and 3-NP (model of HD) in PC12 neuronal cell cultures

The significant clear beneficial effect of amiodarone on different neurodegenerative diseases as demonstrated by the cell and animal models encouraged the inventors to examine the feasibility of using other antiarrhythmic drugs for the treatment of different neurodegenerative disorders. PC-12 cells, exposed to AD-related amyloid neurotoxicity, as well as to 3-NP (HD model), were treated with different concentrations of sotalol (5-100μM). As can be seen in Figure 11, sotalol at a concentration of 100μM protected PC12 cells against AD-related amyloid neurotoxicity. While the cell viability was reduced to 64% in the presence of Aβ (25-35) peptide, treatment with 100μM sotalol rescued the cells, as shown by the significant increase in cell viability to 84%(p=0.06) (Fig. 11A). Similarly, neuroprotective effects were noticed under HD-related 3-NP toxicity: cell viability was increased from 48% (without treatment) to 66%, 68% and 80% when treated with 5μM, 50μM and 100Mm sotalol, respectively (p=0.02) (Fig. 11B).

Neuroprotection against MPTP and 6-OHDA (models of PD), and glutamate (model of ALS) in PC12 neuronal cell is currently examined. PC-12 cells, exposed to MPTP and 6-OHDA (models of PD), as well as to glutamate (model of ALS) are treated with different concentrations of sotalol (5-100μM). Cell viability examined and reflects the neuroprotective ability of sotalol.

Example 6

Flecainide exhibits neuroprotection against Aβ (model of AD), and 3-NP (model of HD) in PC12 neuronal cell cultures

The inventors next examined the potential neuroprotective effect of an additional antiarrhythmic drug, the flecainide, using the PC-12 cell

model. As shown in Figure 12A, flecainide at a concentration of 50-200μM protected PC12 cells against AD-related amyloid neurotoxicity. While the cell viability was reduced to 48% in the presence of Aβ (25-35) peptide, treatment with 50μM, 100μM and 200μM flecainide rescued the cells, as reflected by increase (yet not reaching a statistical significance) in cell viability to 98%, 84% and 84%, respectively. Similarly, as demonstrated by Figure 12B, neuroprotective effects were noticed under HD-related 3-NP toxicity: cell viability was increased from 48% (without treatment) to 88%, 82 and 83% when treated with 50μM, 100μM and 200 μM flecainide, respectively (p=0.06).

Neuroprotection against MPTP and 6-OHDA (models of PD), and glutamate (model of ALS) in PC12 neuronal cell is currently examined. PC-12 cells, exposed to MPTP and 6-OHDA (models of PD), as well as to glutamate (model of ALS) are treated with different concentrations of flecainide (50-200μM). Cell viability is examined and reflects the neuroprotective ability of flecainide.

Example 7

Propafenone showed neuroprotection against Aβ (model of AD), and 3-NP (model of HD) in PC12 neuronal cell cultures

The inventors next examined the potential neuroprotective effect of another antiarrhythmic drug, propafenone, using the PC-12 cell model. As can be seen in Figure. 13, propafenone protected PC12 cells against AD-related amyloid neurotoxicity. While the cell viability was reduced to 45% in the presence of Aβ (25-35) peptide, treatment with 5μM, 50μM and 100μM propafenone rescued the cells, as shown by the significant increase in the cell viability to 83%, over 100%, and 100%, respectively (p=0.02).

Neuroprotection against MPTP and 6-OHDA (models of PD), and glutamate (model of ALS) in PC12 neuronal cell is currently examined. PC-12 cells, exposed to MPTP and 6-OHDA (models of PD), as well as to glutamate (model of ALS) are treated with different concentrations of propafenone (5-200μM). Cell viability is examined and reflects the neuroprotective ability of propafenone.

Example 8

Amiodarone, inhibits acute experimental autoimmune encephalomyelitis (EAE)

The significant neuroprotective effect of amiodarone demonstrated in Examples 2-4, in cell culture and animal models, was further explored by the inventors for investigating its neuroprotective effect in animal models for other diseases of the central nervous system (CNS), including inflammatory diseases affecting the CNS. Therefore, the inventors next examined the effect of amiodarone on experimental autoimmune encephalomyelitis (EAE), an autoimmune disease resulting in demyelination of the white matter in the CNS.

Two mouse models were used for analyzing the potential effect of amiodarone for the treatment of EAE: C57Bl mice immunized with MOG (myelin oligodendrocyte glycoprotein), and SJL mice immunized with PLP (proteolipid protein). As shown by Figure 14A, treatment of C57Bl mice i.p. with 30mg/Kg/day (in the lower range of the concentration reported in use in mice: 30-180mg/Kg/day), starting from the day of immunization (EAE induction), resulted in a reduction of about 50% in disease score. Similar results were noticed using the PLP-EAE model in SJL mice, treated with 30-60mg/Kg/day (Figure 14B). These results indicate for the first time, a neuroptotective effect of amiodarone in animal model for inflammatory diseases of the CNS, and may point to its neuroprotective

potential in patients affected by MS, or by other inflammatory conditions of the CNS, preferably, autoimmne disorders.

While this invention has been described in terms of some specific examples, many modifications and variations are possible. It is therefore understood that within the scope of the appended claims, the invention may be realized otherwise than as specifically described.

Claims

1. A method for the treatment or prevention of a pathologic disorder selected from neurodegenerative disorders and autoimmune-related disorders, in a subject in need thereof, which method comprises the step of administering to said subject a therapeutically effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof.

2. The method according to claim 1, wherein said pathologic disorder is a neurodegenerative disorder selected from a protein misfolding disorder, an amyloid disease, taupathy or a prion disease.

3. The method according to claim 2, wherein said neurodegenerative disorder is selected from the group of Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, Pick's disease, fronto temporal dementia, cortico-basal degeneration and progressive supranuclear palsy, Spongiform encephalopathies, Scrapie, mad cow disease and Bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru.

4. The method according to claim 3, wherein said any one of an antiarrhythmic agent and a cholesterol absorption inhibitor agent exert neuroprotective effect and improves neuronal survival upon exposure to neurotoxic agents.

5. The method according to claim 4, wherein said neurotoxic agent is any one of cholesterol, preferably free cholesterol, Aβ peptide, in particular the peptide of SEQ ID No:1, alpha-synuclein, huntingtin, prion protein, 3-

NP (3-nitropropionic acid), MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, 6OHDA (6-hydroxydopamine) and Glutamate.

6. The method according to claim 1, wherein said pathologic disorder is an autoimmune disorder selected from multiple sclerosis and acute disseminating encephalomyelitis.

7. The method according to any one of claims 2 and 6, wherein said subject is a mammalian subject.

8. The method according to any one of claims 1, 2, and 6, wherein said method comprises the step of administering to said subject a therapeutically effective amount of an antiarrhythmic agent or of a composition comprising the same.

9. The method according to claim 8, wherein said antiarrhythmic agent is any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

10. The method according to claim 9, wherein said antiarrhythmic agent is amiodarone or any active derivatives thereof.

11. The method according to any one of claims 10 and 11, wherein the active ingredient an antiarrhythmic agent or active derivatives thereof are administered alone, or in combination with other active ingredients that improve the therapeutic effect, whether administered in combination, serially or simultaneously.

12. The method according to claim 11, wherein the other active ingredient is a cholesterol absorption inhibitor agent, preferably, ezetimibe.

13. The method according to any one of claims 1, 2, and 6, wherein said method comprises the step of administering to said subject a therapeutically effective amount of a cholesterol absorption inhibitor agent or of a composition comprising the same.

14. The method according to claim 13, wherein said cholesterol absorption inhibitor agent is ezetimibe.

15. The method according to claim 14, wherein the active ingredient is administered alone, or in combination with other active ingredient/s that improve the therapeutic effect, whether administered in combination. serially or simultaneously.

16. The method according to claim 15, wherein the other active ingredient is an antiarrhythmic agent.

17. The method according to claim 16, wherein said antiarrhythmic agent is any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

18. The method according to claim 1, wherein said administering step comprises oral, intravenous, intramuscular, subcutaneous, intraperitoneal, parenteral, transdermal, intravaginal, intranasal, mucosal, sublingual, topical, rectal or subcutaneous administration, or any combination thereof.

19. Use of an antiarrhythmic agent and/or a cholesterol absorption inhibitor agent in the preparation of a pharmaceutical composition for the treatment or prevention of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune related disorder, in a subject in need thereof.

20. The use according to claim 19, wherein said pathologic disorder is a neurodegenerative disorder selected from a protein misfolding disorder, an amyloid disease, taupathy or a prion disease.

21. The use according to claim 20, wherein said neurodegenerative disorder is selected from the group of Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, Pick's disease, fronto temporal dementia, cortico-basal degeneration, progressive supranuclear palsy, Spongiform encephalopathies, Scrapie, mad cow disease and Bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome and Kuru.

22. The use according to claim 19, wherein said pathologic disorder is an autoimmune disorder selected from multiple sclerosis and acute disseminating encephalomyelitis.

23. The use according to any one of claims 20 and 22, wherein said subject is a mammalian subject.

24. The use according to any one of claims 19, 20, and 22, of an antiarrhythmic agent in the preparation of pharmaceutical composition for the treatment or prevention of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune-related disorder, in a subject in need thereof.

25. The use according to claim 24, wherein said antiarrhythmic agent is any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

26. The use according to claim 25, wherein said antiarrhythmic agent is amiodarone or any physiologically acceptable active derivatives thereof.

27. The use according to any one of claims 19, 20, and 22, of a cholesterol absorption inhibitor agent in the preparation of pharmaceutical composition for the treatment or prevention of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune related disorder, in a subject in need thereof.

28. The use according to claim 27, wherein said cholesterol absorption inhibitor agent is ezetimibe.

29. A method for protection of neuronal cells from a neurodegenerative process comprising the step of contacting said cells with a neuroprotective effective amount of any one of an antiarrhythmic agent, a cholesterol absorption inhibitor agent, a composition comprising the same or any combinations thereof.

30. The method according to claim 29, wherein said neurodegenerative process is caused by a neurodegenerative disorder in a mammalian subject, said disorder is selected from a protein misfolding disorder, an amyloid disease, taupathy or a prion disease.

31. The method according to claim 30, wherein said neurodegenerative disorder is selected from the group of Alzheimer's disease, Parkinson's disease, ALS (Amyotrophic Lateral Sclerosis), Huntington's disease, Pick's disease, fronto temporal dementia, cortico-basal degeneration, progressive supranuclear palsy, Spongiform encephalopathies, Scrapie, mad cow disease and Bovine spongiform encephalopathy, Creutzfeldt-Jakob

32. The method according to claim 29, wherein said neurodegenerative process is caused by exposure of said cells to a neurotoxic agent.

33. The method according to claim 32, wherein said neurotoxic agent is any one of cholesterol, preferably free cholesterol, Aβ peptide, in particular the peptide of SEQ ID No:1, alpha-synuclein, huntingtin, prion protein, 3-NP (3-nitropropionic acid), MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, 6OHDA (6-hydroxydopamine) and Glutamate.

34. A method for protection of neuronal cells from a neurodegenerative process according to claim 29, wherein said method comprises the step of contacting said cells with a neuroprotective effective amount of an antiarrhythmic agent or of a composition comprising the same.

35. The method according to claim 34, wherein said antiarrhythmic agent is any one of amiodarone, sotalol, flecainide, propafenone and any physiologically acceptable active derivatives thereof.

36. The method according to claim 35, wherein said antiarrhythmic agent is amiodarone or any active derivatives thereof.

37. A method for protection of neuronal cells from a neurodegenerative process according to claim 29, wherein said method comprises the step of contacting said cells with a neuroprotective effective amount of a cholesterol absorption inhibitor agent or of a composition comprising the same.

38. The method according to claim 37, wherein said cholesterol absorption inhibitor agent is ezetimibe.

39. A method of preparing a therapeutic composition for the treatment of a pathologic disorder selected from a neurodegenerative disorder and an autoimmune related disorder, in a mammalian subject, which method comprises the steps of:

a. providing any one of an antiarrhythmic agent and a cholesterol absorption inhibitor agent; and

b. admixing said antiarrhythmic agent or said cholesterol absorption inhibitor agent with at least one of a pharmaceutically acceptable carrier, diluent, excipient and/or additive.

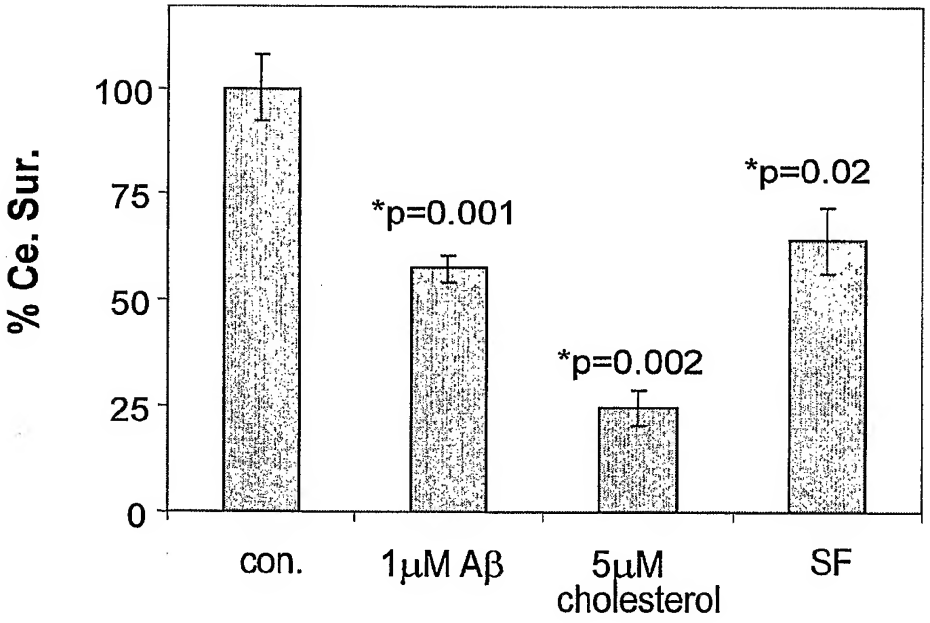

% Ce. Sur.
100
75
50
25
0
*p=0.001
*p=0.002
*p=0.02
con.
1μM Aβ
5μM cholesterol
SF

Fig. 1A

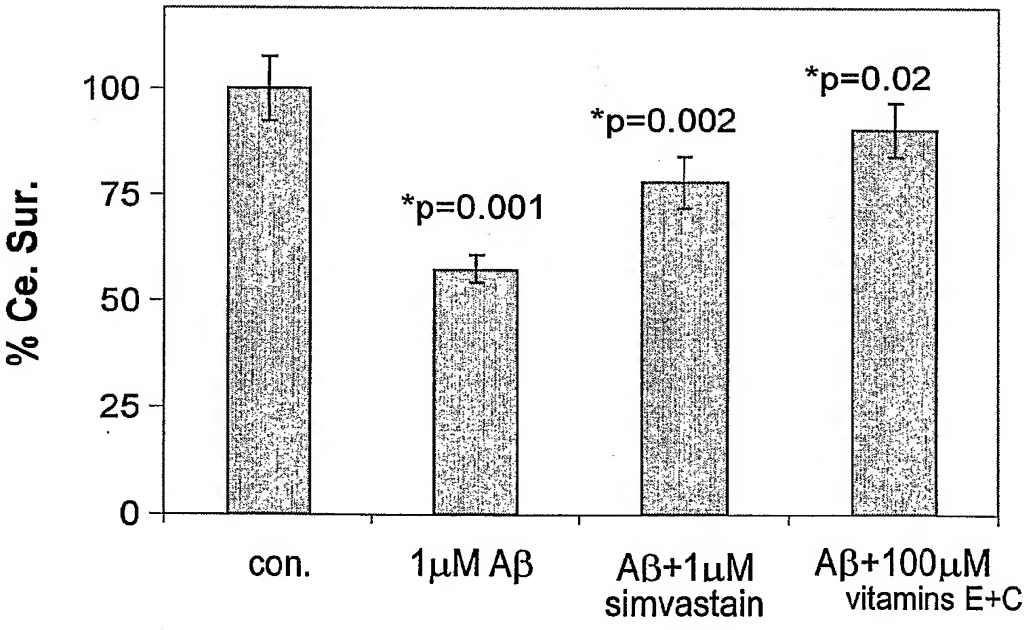

% Ce. Sur.
100
75
50
25
0
*p=0.001
*p=0.002
*p=0.02
con.
1μM Aβ
Aβ+1μM simvastain
Aβ+100μM vitamins E+C

Fig. 1B

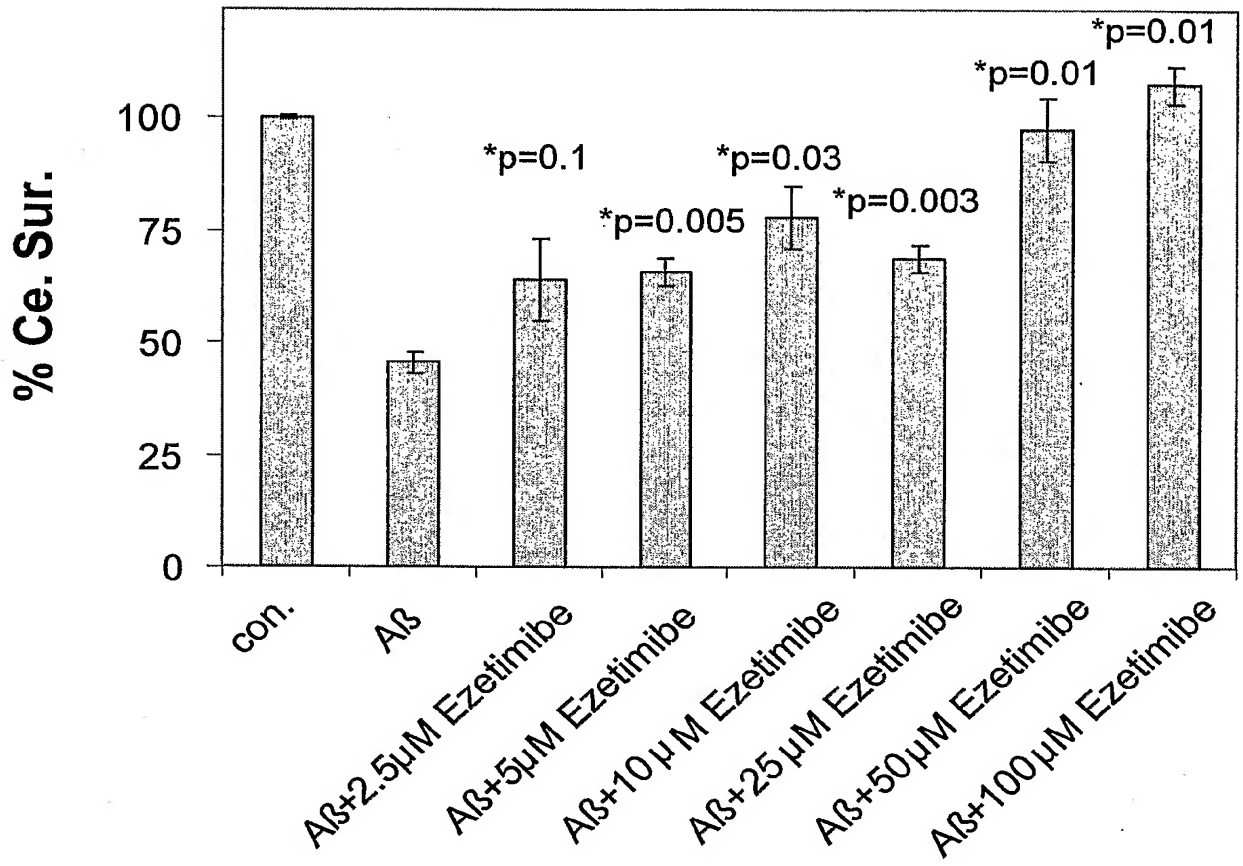

% Ce. Sur.
100
75
50
25
0
*p=0.1
*p=0.005
*p=0.03
*p=0.003
*p=0.01
*p=0.01
con.
Aß
Aß+2.5µM Ezetimibe
Aß+5µM Ezetimibe
Aß+10µM Ezetimibe
Aß+25µM Ezetimibe
Aß+50µM Ezetimibe
Aß+100µM Ezetimibe

Fig. 2

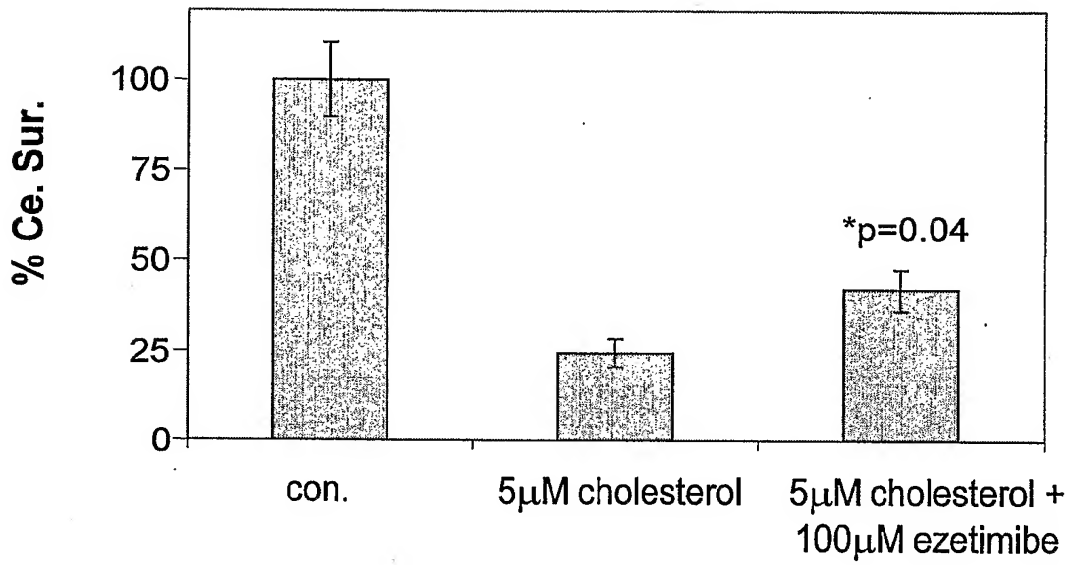

% Ce. Sur.
100
75
50
25
0
*p=0.04
con.
5μM cholesterol
5μM cholesterol +
100μM ezetimibe

Fig. 3

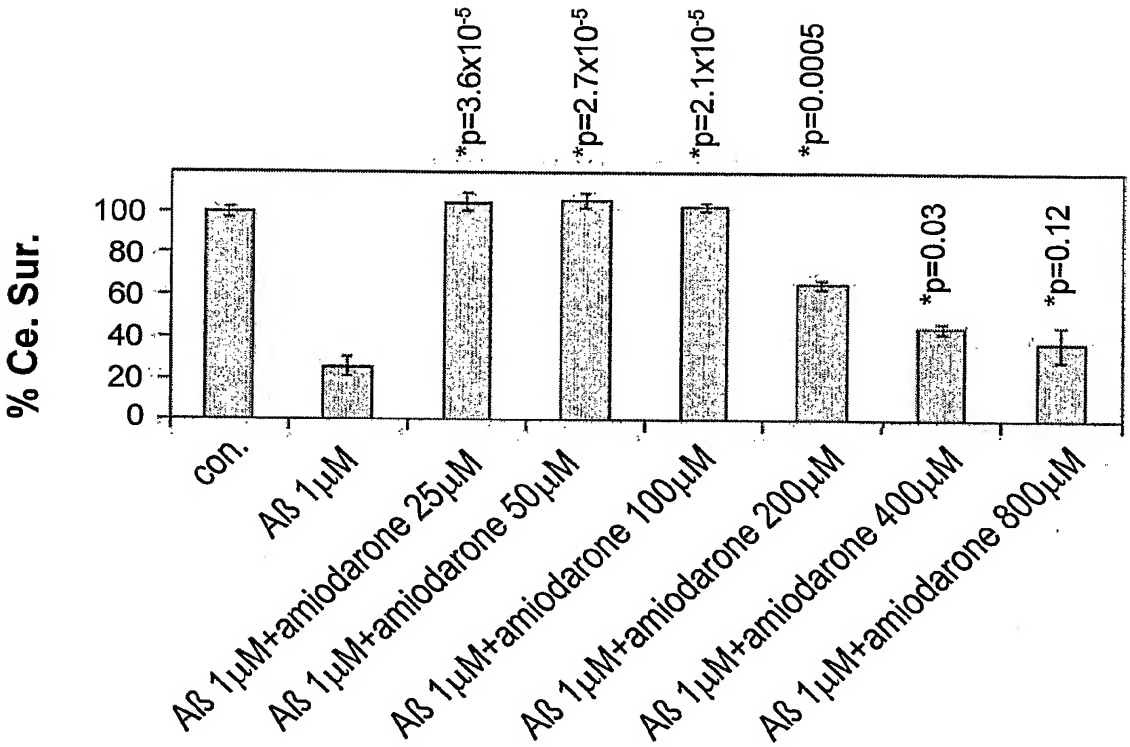

% Ce. Sur.
100
80
60
40
20
0
*p=3.6x10-5
*p=2.7x10-5
*p=2.1x10-5
*p=0.0005
*p=0.03
*p=0.12
con.
Aß 1µM
Aß 1µM+amiodarone 25µM
Aß 1µM+amiodarone 50µM
Aß 1µM+amiodarone 100µM
Aß 1µM+amiodarone 200µM
Aß 1µM+amiodarone 400µM
Aß 1µM+amiodarone 800µM

Fig. 4

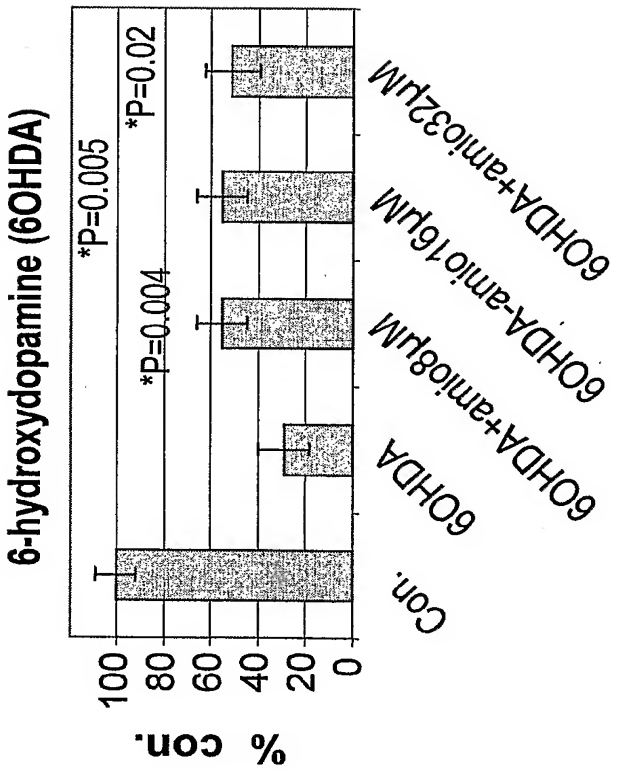

6-hydroxydopamine (6OHDA)
*P=0.005
*P=0.02
*P=0.004
% con.
100
80
60
40
20
0
Con.
6OHDA
6OHDA+amio8µM
6OHDA-amio16µM
6OHDA+amio32µM

Fig. 5A

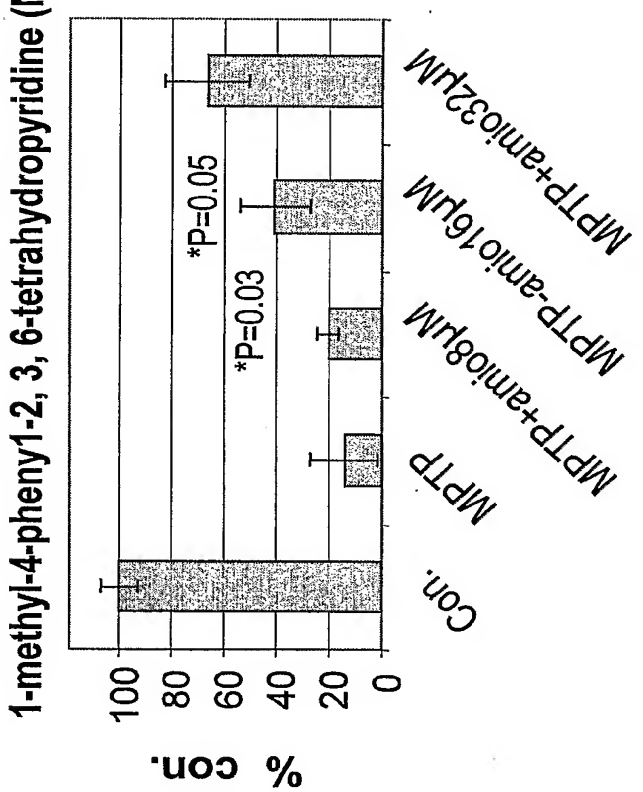

1-methyl-4-pheny1-2, 3, 6-tetrahydropyridine (MPTP)
*P=0.05
*P=0.03
% con.
100
80
60
40
20
0
Con.
MPTP
MPTP+amio8µM
MPTP-amio16µM
MPTP+amio32µM

Fig. 5B

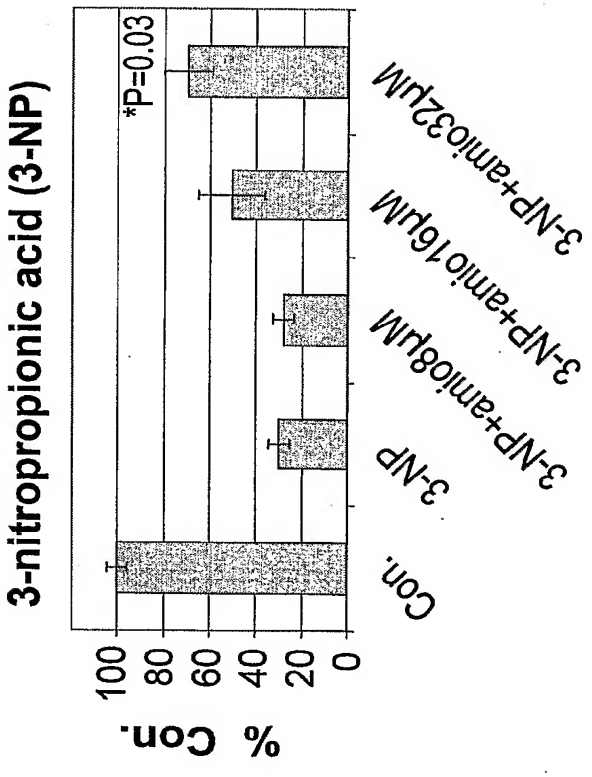

3-nitropropionic acid (3-NP)
*P=0.03
% Con.
100
80
60
40
20
0
Con.
3-NP
3-NP+amio8µM
3-NP+amio16µM
3-NP+amio32µM

Fig. 5C

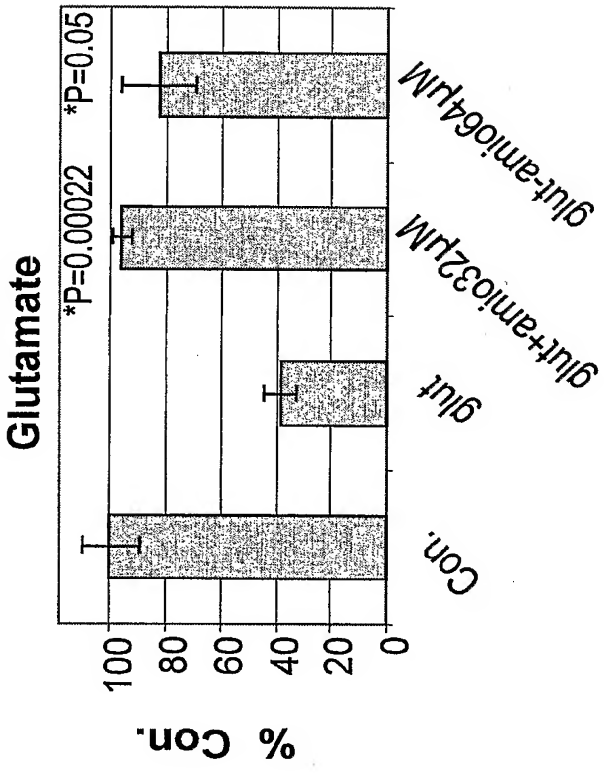

Glutamate
*P=0.00022
*P=0.05
% Con.
100
80
60
40
20
0
Con.
glut
glut+amio32µM
glut-amio64µM

Fig. 5D

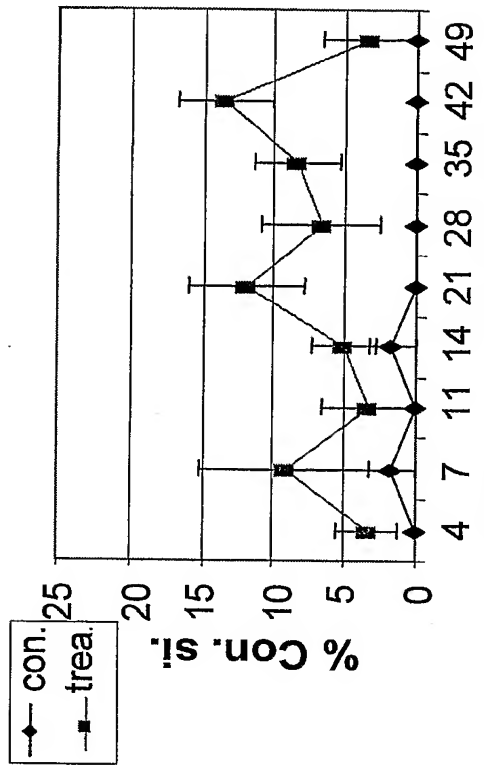


Fig. 6B

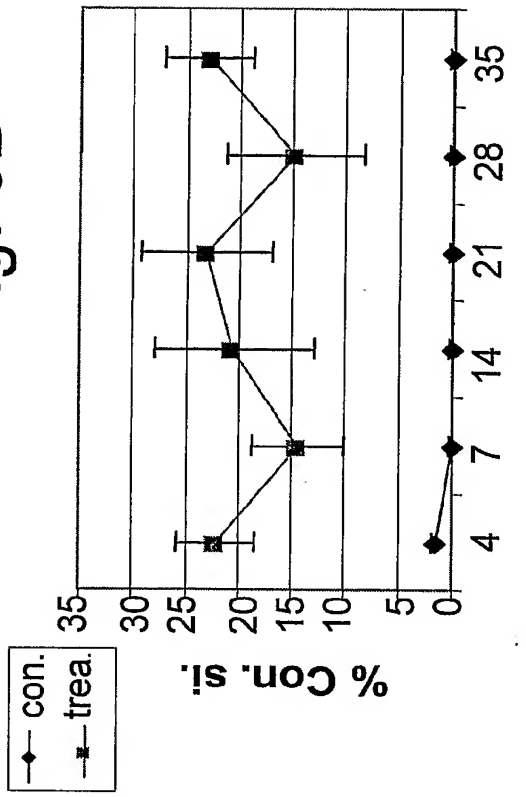


Fig. 6D

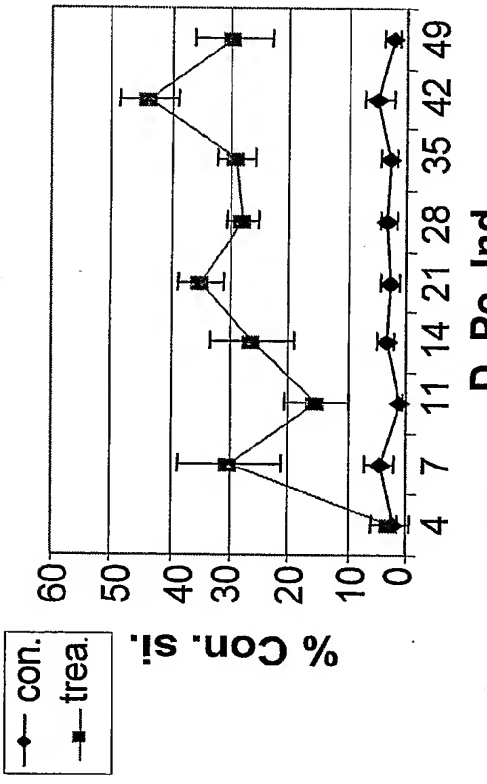


Fig. 6A

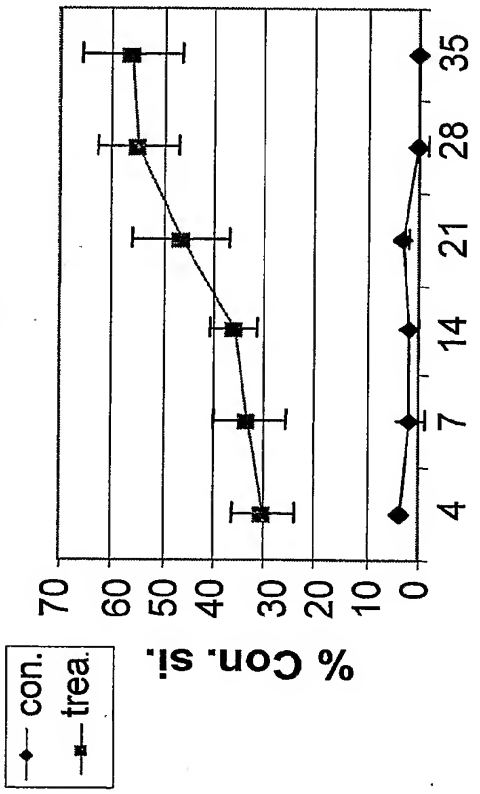


Fig. 6C

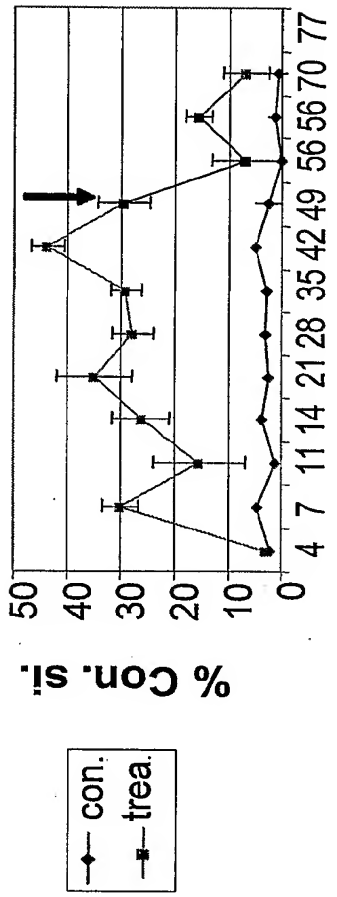

% Con. si.
0
10
20
30
40
50
4 7 11 14 21 28 35 42 49 56 56 70 77
D. Po. Ind.
con.
trea.

Fig. 7A

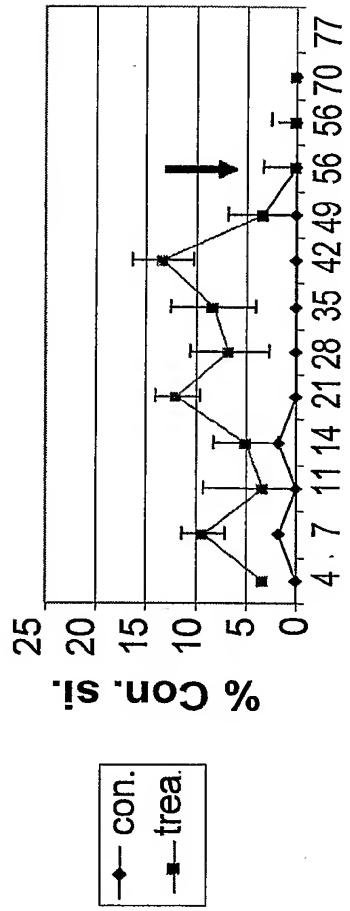

% Con. si.
0
5
10
15
20
25
4 7 11 14 21 28 35 42 49 56 56 70 77
D. Po. Ind.
con.
trea.

Fig. 7B

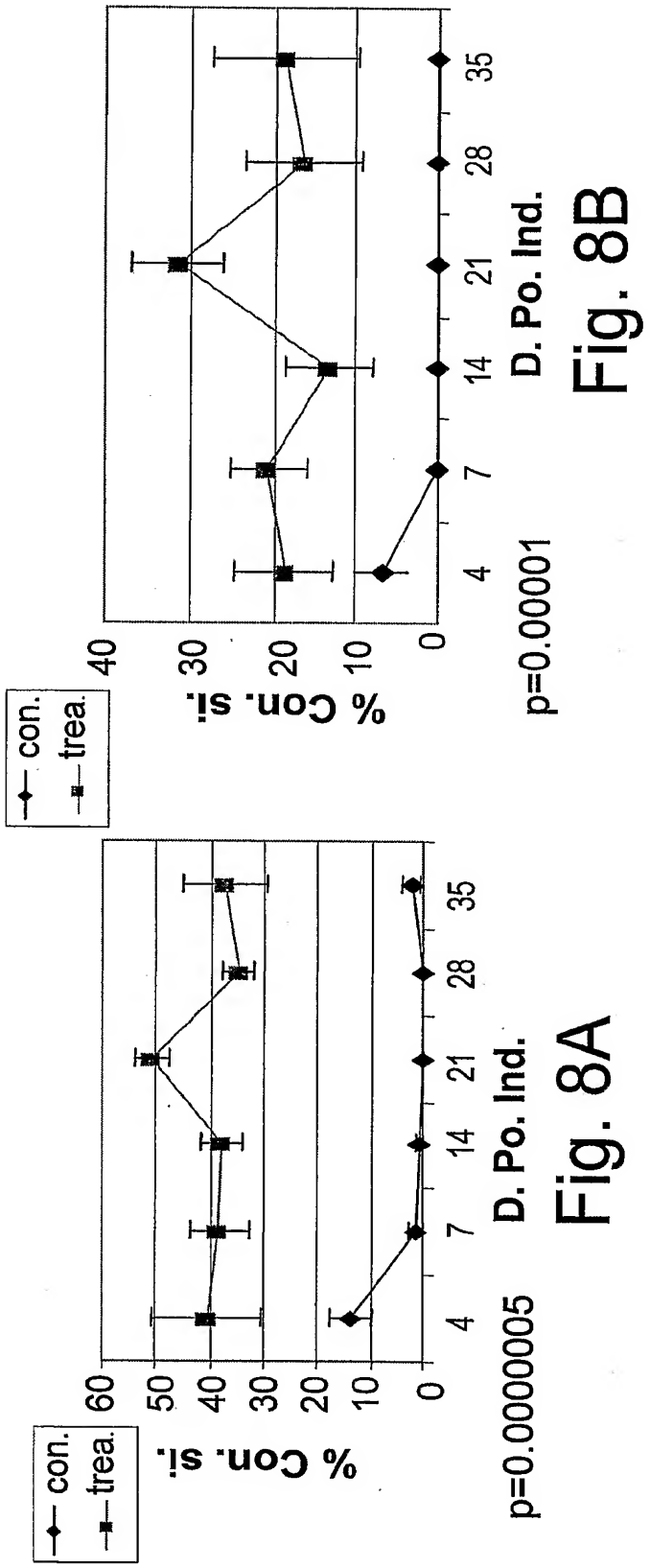


Fig. 8A

Fig. 8B

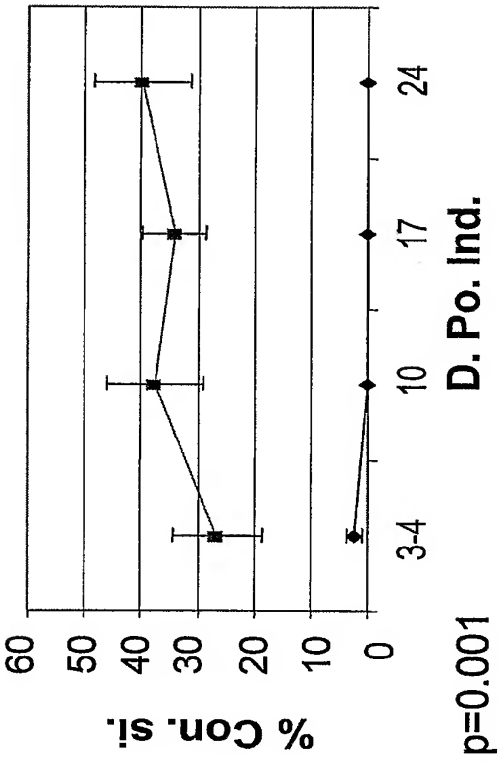


Fig. 8C

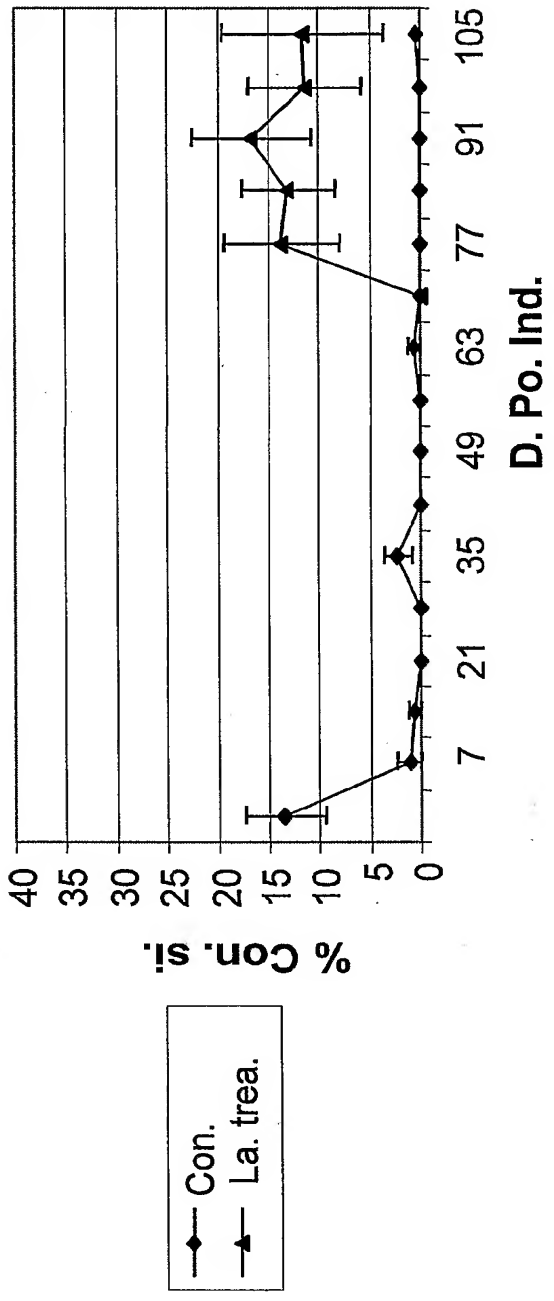

Con.
La. trea.
% Con. si.
0
5
10
15
20
25
30
35
40
7
21
35
49
63
77
91
105
D. Po. Ind.

Fig. 9

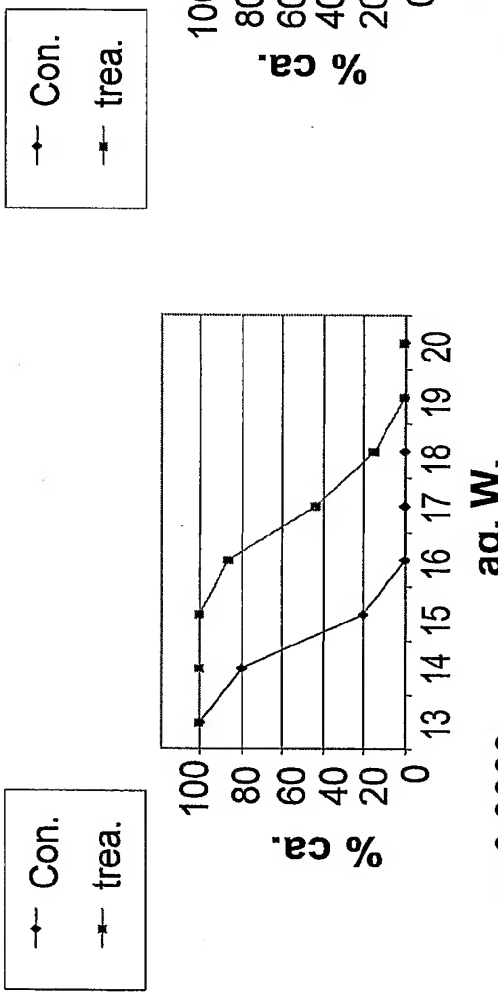


Fig. 10A

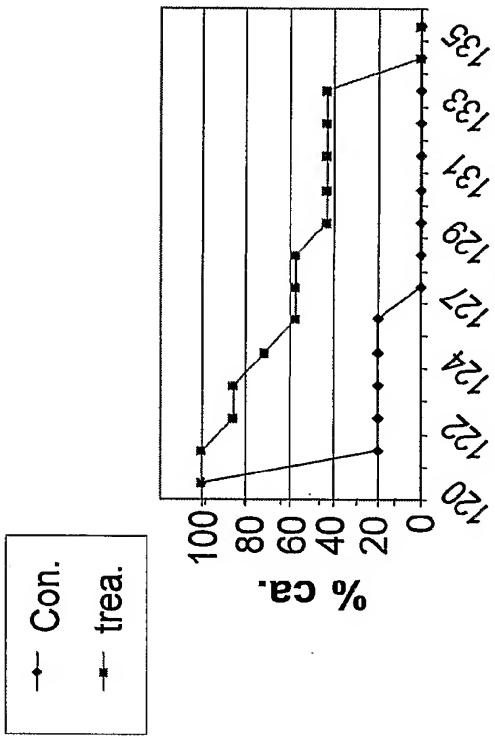


Fig. 10B

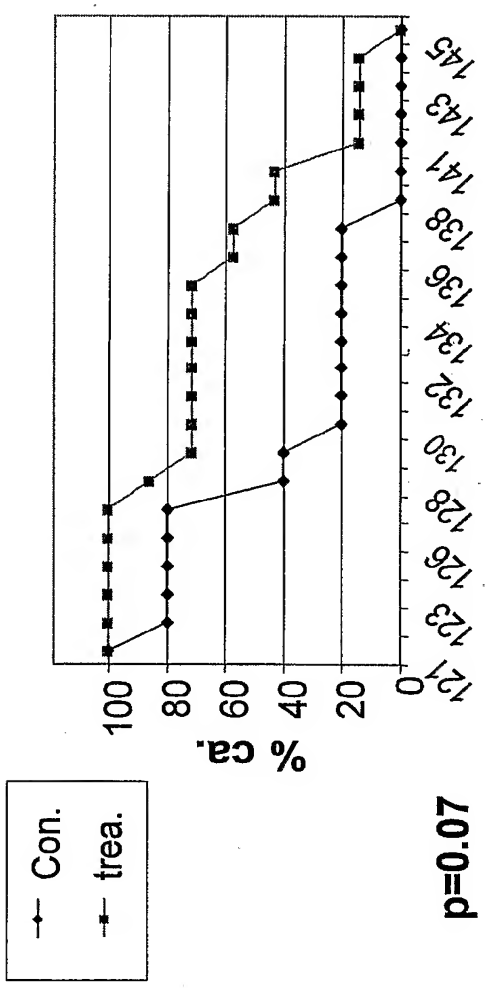


Fig. 10C

Fig. 11A

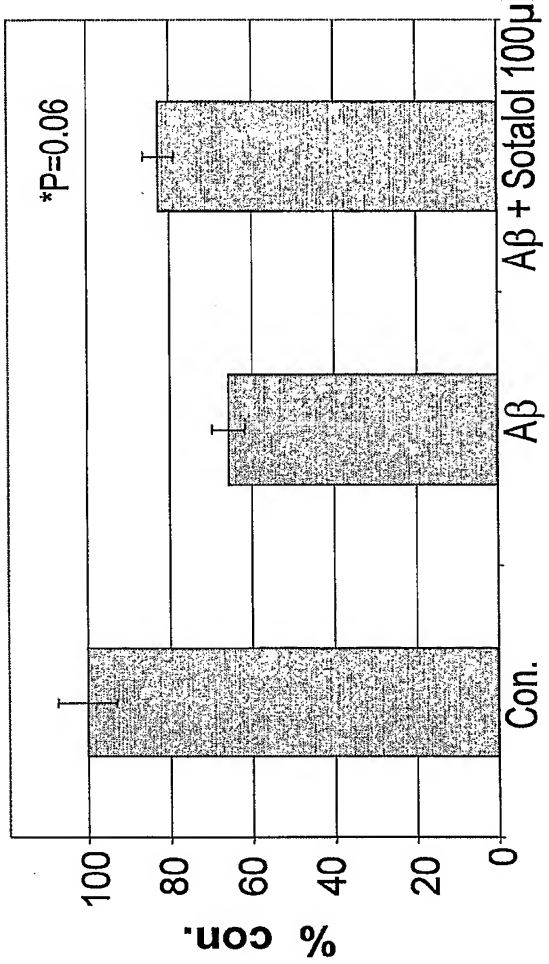


Fig. 11B

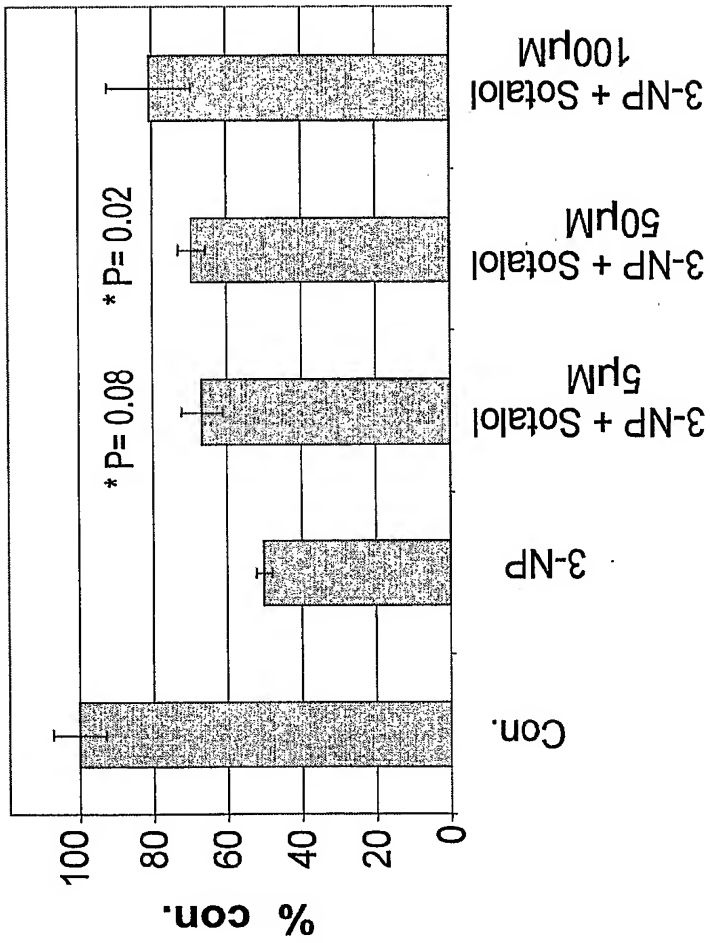


Fig. 12A

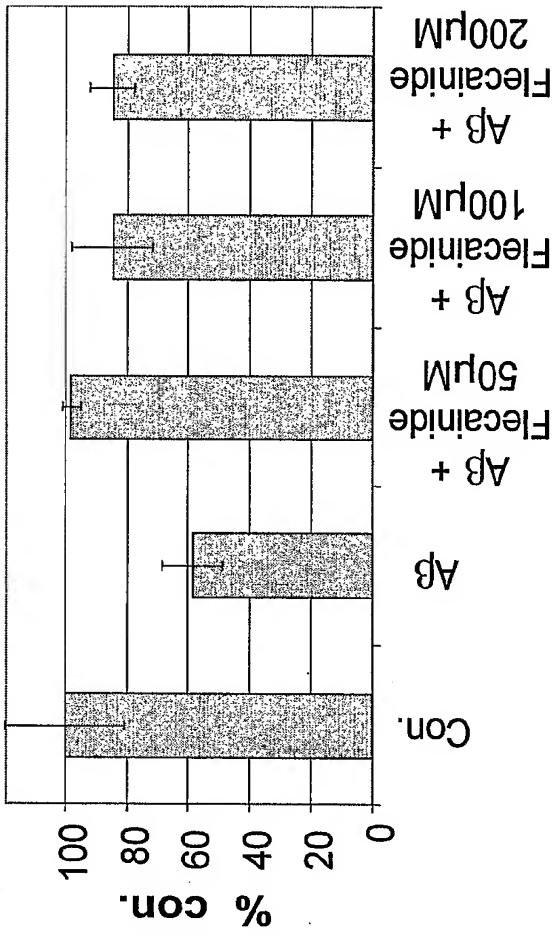
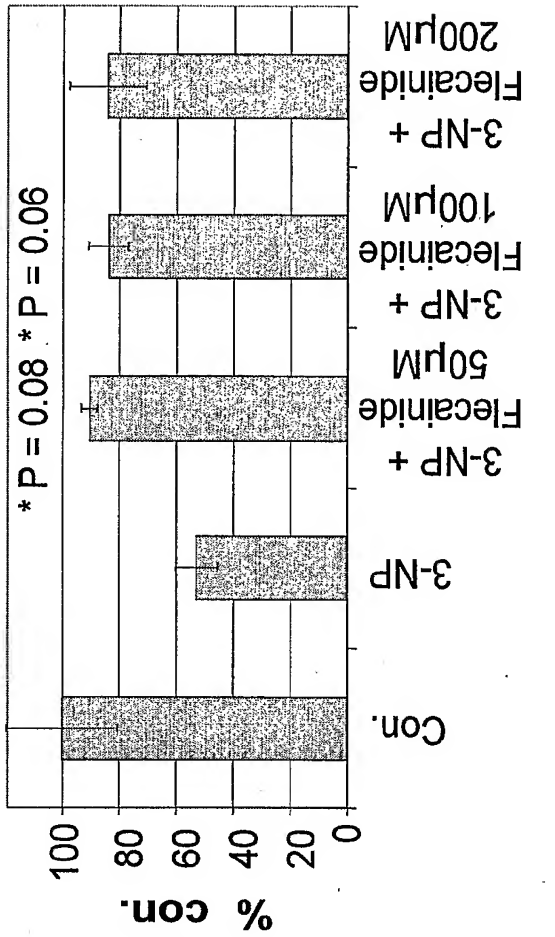


Fig. 12B



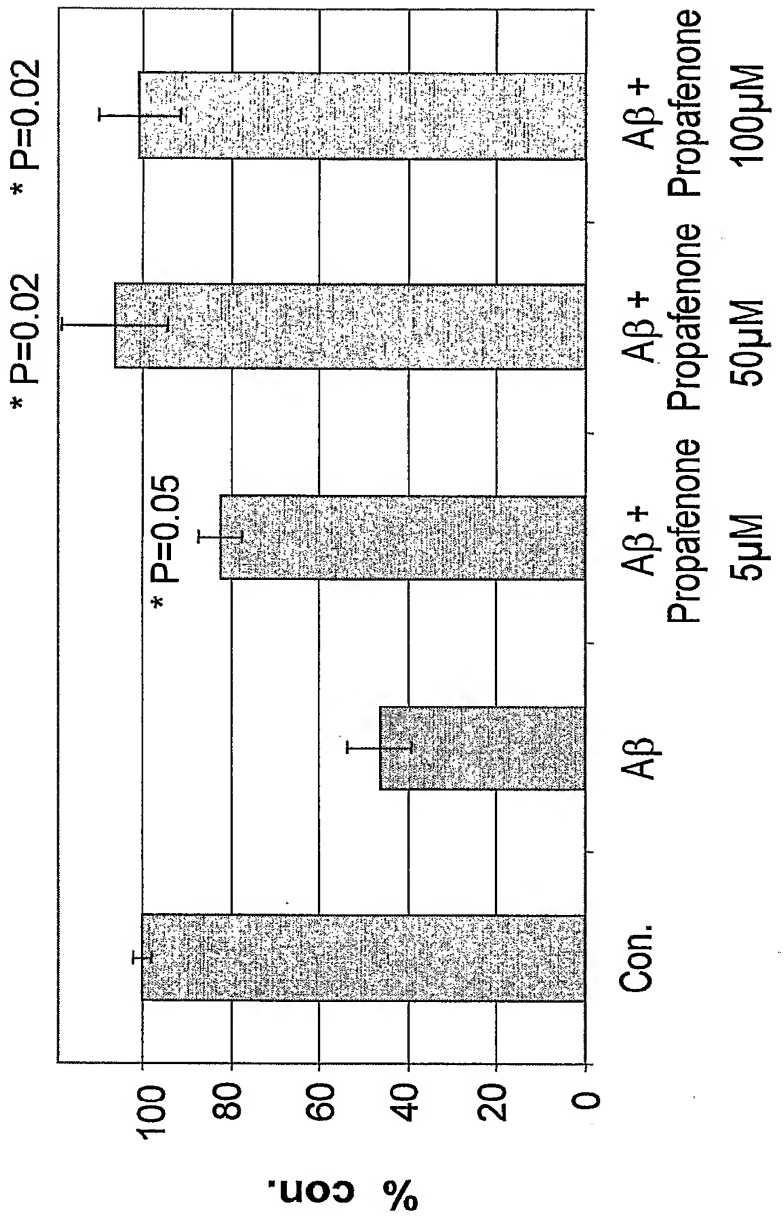

* P=0.02
* P=0.02
* P=0.05
100
80
60
40
20
0
% con.
Con.
Aβ
Aβ + Propafenone 5μM
Aβ + Propafenone 50μM
Aβ + Propafenone 100μM

Fig. 13

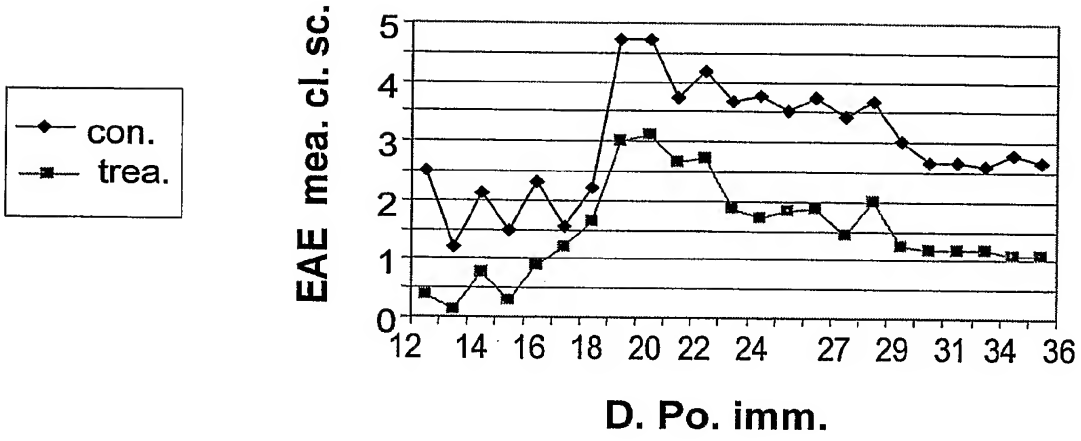


Fig. 14A

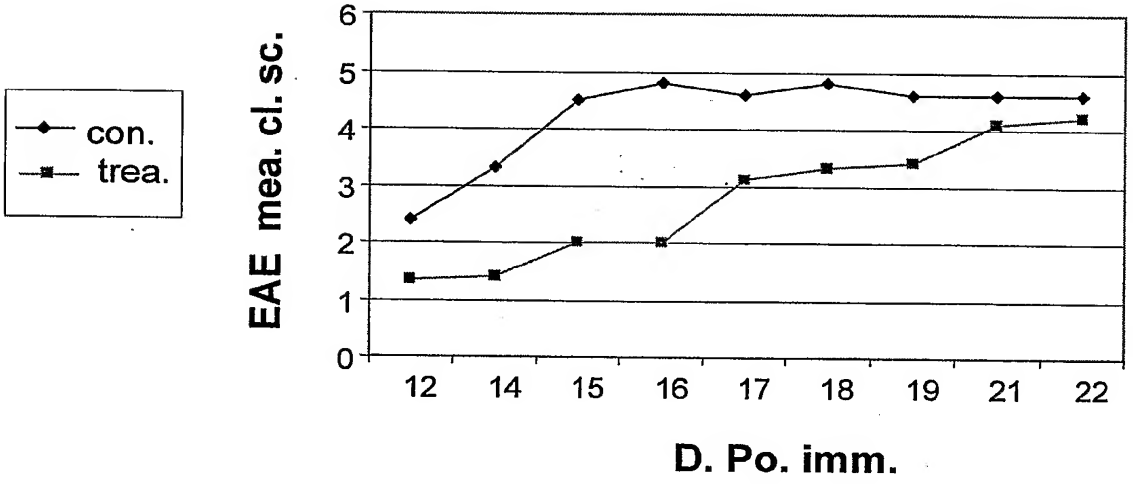


Fig. 14B

18299WO.ST25
SEQUENCE LISTING

<110> Hadasit Medical Research Services & Development Ltd.

<120> ANTI-ARRHYTHMIC DRUGS AND CHOLESTEROL ABSORPTION INHIBITORS AS NEUROPROTECTIVE AGENTS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

<130> 18299-WO-04

<150> IL 166149
<151> 2005-01-05

<160> 1

<170> PatentIn version 3.3

<210> 1
<211> 10
<212> PRT
<213> Homo sapiens

<400> 1

Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
1 5 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2006/000027

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/343 A61K31/18 A61K31/445 A61K31/49 A61K31/397
A61P25/28 A61P37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/043456 A (SCHERING CORPORATION) 27 May 2004 (2004-05-27) page 7 claims 10,11,14	1-3,6,7, 13-21, 24, 27-31, 37-39
X	WO 03/082192 A (SMITHKLINE BEECHAM CORPORATION; THOMPSON, SCOTT, K; FRAZEE, JAMES, S;) 9 October 2003 (2003-10-09) page 1, line 26 - page 2, line 3 page 23, line 19 - line 30	1-7,13, 19-24, 27, 29-34, 37,39

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2006	12/06/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Albayrak, T

3

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STYS PETER K: "Axonal degeneration in multiple sclerosis: is it time for neuroprotective strategies?" ANNALS OF NEUROLOGY. MAY 2004, vol. 55, no. 5, May 2004 (2004-05), pages 601-603, XP002376095 ISSN: 0364-5134 page 601, left-hand column	1-3,6-9, 11,12, 18,19, 22-25, 29,30, 34,35,39
X	BECHTOLD DAVID A ET AL: "Axonal protection using flecainide in experimental autoimmune encephalomyelitis" ANNALS OF NEUROLOGY, vol. 55, no. 5, May 2004 (2004-05), pages 607-616, XP002376096 ISSN: 0364-5134 page 611, right-hand column page 615, left-hand column	1-3,6-9, 11,12, 18,19, 22-25, 29,30, 34,35,39
P,X	VAN HEYNINGEN CHARLES: "Drug-induced acute autoimmune hepatitis during combination therapy with atorvastatin and ezetimibe." ANNALS OF CLINICAL BIOCHEMISTRY. SEP 2005, vol. 42, no. Pt 5, September 2005 (2005-09), pages 402-404, XP009064678 ISSN: 0004-5632 page 402 - page 403	1,12-19, 27,28,39
X	GUDBJORNSSON B ET AL: "PAINFUL AUTOIMMUNE THYROIDITIS OCCURRING ON AMIODARONE THERAPY" ACTA MEDICA SCANDINAVICA, vol. 221, no. 2, 1987, pages 219-220, XP009064677 ISSN: 0001-6101 page 220	1,8-12, 17-19, 24-26,39
X	MATSUI SHINOBU ET AL: "Amiodarone minimizes experimental autoimmune myocarditis in rats." EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 469, no. 1-3, 23 May 2003 (2003-05-23), pages 165-173, XP002376097 ISSN: 0014-2999 page 172, left-hand column	1,8-12, 17-19, 24-26,39

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2006/000027

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WERNER E G ET AL: "PARKINSONISM AND AMIODARONE THERAPY" ANNALS OF NEUROLOGY, vol. 25, no. 6, 1989, pages 630-632, XP009064676 ISSN: 0364-5134 page 631, right-hand column - page 632, left-hand column	1-3, 7-12, 18-21, 23-26, 29-31, 34-36,39
X	MATSUI AKIKO ET AL: "Prediction of catalepsies induced by amiodarone, aprindine and procaine: Similarity in conformation of diethylaminoethyl side chain" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 287, no. 2, November 1998 (1998-11), pages 725-732, XP002376098 ISSN: 0022-3565 page 725, left-hand column	1-3, 7-12, 18-21, 23-26, 29-31, 34-36,39
X	MADIGAND M ET AL: "[Myoclonic encephalopathy probably attributable to propafenone]" PRESSE MÉDICALE (PARIS, FRANCE : 1983) 26 MAR 1988, vol. 17, no. 11, 26 March 1988 (1988-03-26), page 538, XP009064901 ISSN: 0755-4982 the whole document	1-3,7-9, 11,12, 18-21, 23-25, 29-31, 34,35,39
X	JEANDEL C ET AL: "[Myoclonic encephalopathy caused by propafenone]" THÉRAPIE. 1990 MAR-APR, vol. 45, no. 2, March 1990 (1990-03), pages 161-162, XP009064900 ISSN: 0040-5957 the whole document	1-3,7-9, 11,12, 18-21, 23-25, 29-31, 34,35,39
X	FUNCK-BRENTANO C: "Pharmacokinetic and pharmacodynamic profiles of d-sotalol and d, l - sotalol" EUROPEAN HEART JOURNAL, THE EUROPEAN SOCIETY OF CARDIOLOGY, vol. 14, no. SUPPL H, 1993, pages 30-35, XP002109598 ISSN: 0195-668X the whole document	39

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IL2006/000027

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004043456 A	27-05-2004	AU 2003291719 A1	03-06-2004
		CA 2504878 A1	27-05-2004
		CA 2504916 A1	27-05-2004
		EP 1562582 A1	17-08-2005
		EP 1560579 A1	10-08-2005
		WO 2004043457 A1	27-05-2004
WO 03082192 A	09-10-2003	AU 2003223340 A1	13-10-2003
		EP 1490047 A2	29-12-2004
		JP 2005521705 T	21-07-2005